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Looking for a bottleneck: Assessment of factors influencing post-stocking survival of advanced fingerling Walleye Sander vitreus

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Looking for a bottleneck: Assessment of factors influencing post-stocking survival of advanced fingerling Walleye *Sander vitreus*

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

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DEDICATION

This dissertation is dedicated to my family, your endless support and encouragement has played a crucial role in my accomplishments, thank you!

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ABSTRACT

Walleye *Sander vitreus* is a highly valued sportfish in North America. In 2001, 3.8 million anglers spent approximately 51.9 million days angling for Walleye (USFWS-USCB 2002). The popularity of Walleye has resulted in situations where demand exceeds supply, which has led to the development and implementation of stocking programs across the United States and Canada to enhance fishing opportunities (Lathrop et al. 2002), rebuild depleted stocks (Johnson et al. 1996; Li et al. 1996), and mitigate poor year classes from variable natural recruitment (Mitzner 1992; Johnson et al. 1996; Jennings et al. 2005; Logsdon et al. 2016; Reed and Staples 2017). However, mortality rates of stocked fishes can vary widely (27-95%; Stein et al. 1981; Buckmeier et al. 2005; Freedman et al. 2012; Weber et al. 2020) and small changes in survival can result in large differences in year-class strength and success of stocking initiatives.

Numerous biotic and abiotic factors can influence survival during early life stages of fish, such as transportation and stocking practices (Forsberg et al. 2001; Barton et al. 2003), predation (Santucci and Wahl 1993; Buckmeier et al. 2005; Thompson et al. 2016), available forage (Johnson et al. 1996; Hoxmeier et al. 2006), competition (Le Pape and Bonhommeau 2015; Chase et al. 2016), fish origin (Kellison et al. 2000; Jonsson and Jonsson 2003; Pollock et al. 2007), body size (Litvak and Leggett 1992; Meekan et al. 2006; Grausgruber and Weber *in press*), and water temperatures (Akimova et al. 2016; Wagner et al. 2017). Furthermore, the aforementioned factors do not act independently of each other, making it challenging to determine their importance. The growth-predation hypothesis predicts that selective mortality should decline as individuals grow and increase in size (Anderson 1988). Increases in size are also associated with decreased predation risk (Post and Evans 1989; Miranda and Hubbard 1994), where larger body size can reduce the chances of predation due to improved

maneuverability and swimming speed (Videler 1993). The argument of "bigger-is-better" (Butler 1988; Miller et al. 1988; Litvak and Leggett 1992) has led hatcheries to raise progressively larger fingerling Walleye (Halverson 2008). However, hatchery production is an expensive and labor-intensive process, where production costs are generally positively related to rearing duration and fish size (Wedemeyer 2001). Therefore, it is advantageous to evaluate factors hypothesized to limit post-stocking Walleye survival (e.g., effects of transport duration and handling practices as well as post-stocking predation and starvation) to assess whether rearing larger fingerling Walleye (hereafter referred to as Walleye) is justifiable. The objectives of this dissertation included 1) evaluating relationships between Walleye transport duration with changes in water chemistry parameters, Walleye physiology, and short-term (48 hr) mortality; 2) evaluating whether consumed Walleye total length was related to predator total length, predator gape height, or the probability of predation, as well as assessed whether length distributions varied among stocked, recaptured, and consumed Walleye, and estimate cumulative consumption for up to two months by a suite of piscivores; 3) evaluating diet composition and shifts of stocked Walleye; and 4) use mark-recapture techniques to evaluate the influence of predation, stocking environment, and Walleye physical characteristics on apparent weekly survival of Walleye from stocking until ice cover.

For my first objective, I used two approaches (i.e., field and experimental evaluation) to evaluate relationships between Walleye transport duration with changes in water chemistry parameters, Walleye whole blood glucose and plasma cortisol concentrations, and short-term (48 hr) mortality. For the field evaluation, Walleye were transported between 3.5 to 6 hours and stocked into holding cages located at one of six systems. Water quality and Walleye stress (via whole blood glucose and plasma cortisol) and mortality were evaluated prior to, during, and at 0,

2, 24, and 48 hours post-transportation. During transport, water temperatures generally decreased while carbon dioxide concentrations fluctuated between 2.7 and 22.5 mg/L. Walleye whole blood glucose and plasma cortisol concentrations varied by system and time since transport. Changes in carbon dioxide concentrations were associated with changes in whole blood glucose concentrations. However, cumulative survival rates and plasma cortisol concentrations were not associated with water quality parameters or transportation duration. For the experimental evaluation, Walleye were transported either 0, 0.5, 3, or 5 hours and stress and mortality were evaluated up to 48 hours post-transport. Unlike, the field evaluation, the experimental evaluation used a staggered loading protocol to transport all Walleye on the same truck during the same day and allowed us to keep fish densities in each of the transportation truck compartments consistent throughout the experiment. Furthermore, in the experimental evaluation, I evaluated additional water quality parameters, not included in the field evaluation. In the experimental evaluation, total ammonia nitrogen, carbon dioxide, pH, and water temperature increased with transportation duration while the total alkalinity of the transport water decreased. Plasma cortisol and whole blood glucose concentrations of Walleye transported longer durations took longer to decline relative to those not transported. However, water quality parameters were not associated with changes in whole blood glucose and plasma cortisol concentrations. Despite increases in stress, Walleye mortality was low (2.5%) 48 hours after transportation. Understanding Walleye tolerance to transportation induced stress has the potential to enhance stocking programs by providing the opportunity for managers to make informed Walleye transportation practices.

My second objective was to evaluate predation on Walleye, including whether consumed Walleye total length was related to predator total length, predator gape height, or the probability of predation, as well as assessed if length distributions varied among stocked, recaptured, and

consumed Walleye. I also estimated cumulative consumption by a suite of piscivores (Largemouth Bass *Micropterus salmoides*, Smallmouth Bass *M. dolomieu*, Northern Pike *Esox lucius*, adult Walleye, and Muskellunge *E. masquinongy*) for two months post-stocking. During fall 2015-2017, 301 Walleye were recovered from 3,514 predator stomachs. Quantile regression models (mean = 50^{th} percentile, maximum = 85^{th} percentile, and minimum = 15^{th} percentile) indicated that consumed Walleye total length was not related to predator total length or gape height ($P > 0.05$), but the probability of predation decreased by 0.02 for every 10 mm increase in Walleye total length. Length distributions indicated consumed Walleye were generally smaller, whereas recaptured Walleye tended to be larger than stocked fish. To estimate cumulative consumption of Walleye by Largemouth Bass, Northern Pike, and adult Walleye, I used Schnabel models to estimate predator population size and bioenergetics models to determine the biomass of consumed Walleye from stocking through ice-up (mid- November) during fall 2017 and 2018. Across years and systems, 3,272 predators were collected and 257 Walleye were recovered from predator stomachs. Northern Pike (0.12 Walleye per predator ± 0.32 SD) had the highest proportion of Walleye in their diets followed by Largemouth Bass (0.11 ± 0.30 Walleye per predator) and adult Walleye (0.04 ± 0.20 Walleye per predator). Largemouth Bass, Northern Pike, and adult Walleye collectively consumed between 2.4-27.2% of the stocked Walleye within two months of stocking, with higher proportions of smaller (<220 mm) Walleye in predator diets. In East Okoboji, highest proportions of age-0 Walleye in predator diets generally occurred 14 days after the most recent stocking event with the proportion of age-0 Walleye in predator diets decreasing thereafter. However, in West Okoboji, Northern Pike, adult Walleye, and Largemouth Bass had increasing proportions of age-0 Walleye in diets between stocking events. Collectively, results suggest that local piscivores have the potential to consume more

than 27% of stocked age-0 Walleye within the first two months of stocking and that predation risk generally decreases with Walleye size. Thus, post-stocking predation can negatively influence Walleye stocking success, especially if the sizes of Walleye stocked have high predation risk.

Hatchery propagation techniques, such as pellet-rearing, can result in impaired post-stocking feeding behavior of fishes. My third objective was to evaluate diet composition and diet shifts of Walleye. Specifically, my objective was to compare fall diets of wild and stocked Walleye by evaluating the proportion of empty stomachs as well as proportions of zooplankton, benthic invertebrates, and fish in diets. Percent similarity index values were also used to assess the percent similarity of wild and fingerling diets. In total, 590 Walleye were gastrically lavaged, with wild Walleye making up 9.7% and stocked Walleye making up 90.3% of the samples. The average proportion of empty stomachs differed between wild and stocked Walleye, with stocked Walleye having higher average (± 95% CI) proportions of empty stomachs (0.40 ± 0.10) compared to wild fish (0.15 ± 0.09). Proportion of empty stomachs and proportion of Walleye with zooplankton in diets decreased with days post-stocking. Stocked Walleye had a greater proportion of benthic invertebrates in their diet than wild Walleye whereas wild Walleye consumed more fish relative to stocked Walleye. However, the presence of zooplankton, benthic invertebrates, and fish in wild (TL = 129-215 mm) and fingerling (TL = 94-228 mm) Walleye diets were not related to Walleye total length. Finally, percent similarity index values for wild and stocked Walleye diets were highly variable and ranged from 0.0 to 67.9%. Collectively, my results suggest that fall stocked fingerling Walleye consume different prey items compared to their wild counterparts for up to 49 days post-stocking. Results pertaining to post-stocking Walleye diet composition may influence post-stocking survival.

Numerous factors can independently and dependently influence the survival of stocked Walleye, including environmental conditions, predation, and Walleye total length, condition, and post-stocking diet composition. However, the relative effects of these factors on survival of stocked Walleye are challenging to evaluate and rarely assessed simultaneously. Thus, my fourth objective used mark-recapture techniques to evaluate the influence of predation, stocking environment, and Walleye physical characteristics (total length and condition at time of stocking) on apparent weekly survival from stocking until ice cover. Each fall (2015, 2016, and 2017), roughly 4,000 stocked Walleye were implanted with passive integrated transponder tags, stocked, and recaptured via boat electrofishing. Cormack-Jolly-Seber recapture models estimated that Walleye weekly apparent survival was negatively related to the weekly average proportion of Walleye recovered from predator diets (β = -2.86; 95% credibility interval = -5.69 to -0.17) as well as weekly average water temperatures (β = 0.09; 95% credibility interval = -0.08 to 0.20). At an average fall water temperature, mean Walleye apparent survival was 0.83 (95% credibility interval = 0.75 to 0.91) when no Walleye were recovered from predator diets and declined to 0.54 (95% credibility interval = 0.28 to 0.84) when Walleye comprised 60% of predator diets. Between 41-97% of stocked Walleye were lost to mortality from stocking through ice-up among the three years, indicating that the two months post-stocking is a critical period for survival. Our results suggest that post-stocking Walleye survival is most influenced by post-stocking predation rather than individual Walleye or environmental factors. Thus, strategies that minimize predation following stocking may result in the greatest improvements in Walleye post-stocking survival.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

Historically, the native range of Walleye *Sander vitreus* in the United States extended west from New Hampshire through the Great Lakes basin, southward on the Atlantic slope to North Carolina and west of the Appalachians from the Alabama River system of Georgia to the Tennessee River drainage of Alabama, and from the lower Mississippi River valley to North Dakota (Barton 2011). In Canada, the native range extended from the mouth of the St. Lawrence River west to include the Hudson Bay and Great Lakes drainage, the Saskatchewan River system, and the Great Slave Lake and Mackenzi River (Barton 2011). Walleye stocking has occurred within and outside their native range in all of the lower 48 states (Fuller 2010) in an effort to enhance fishing opportunities (Lathrop et al. 2002), rebuild depleted stocks (Li et al. 1996; Johnson et al. 1996), and mitigate poor year classes from variable natural recruitment (Mitzner 1992; Johnson et al. 1996; Jennings et al. 2005; Logsdon et al. 2016; Reed and Staples 2017). Highly variable recruitment is common in both stocked and non-stocked Walleye populations across North America (Isermann 2007; Nate et al. 2011). Additionally, Walleye recruitment rates across the Midwestern United States have been decreasing (Beard et al. 2003; Hansen et al. 2015). Thus, many state and provincial agencies have developed Walleye stocking programs and post-stocking evaluations to assess stocking success.

Walleye are stocked throughout Canada and the United States at different life stages and sizes (Fenton et al. 1996; Reed and Staples 2017). The smallest size class are fry, which are roughly 9 mm in total length at stocking (Summerfelt 1996; Clapp et al. 1997). The next size class is referred to as small or intensive culture fingerlings (25-50 mm; Koppelman et al. 1992; Clapp et al. 1997; Brooks et al. 2002) followed by large fingerlings (95-100 mm; Clapp et al.

1997; Brooks et al. 2002). Finally, the largest size class of stocked Walleye fingerlings are referred to as advanced fingerlings or extensive-intensive Walleye fingerlings (152-203 mm; Summerfelt 1996; Reed and Staples 2017). Hatchery production of Walleye can be an expensive, labor-intensive process that is related to the size of fish produced. Larger individuals generally have higher survival when stocked compared to smaller individuals but are more expensive to produce (Santucci and Wahl 1993; Santucci et al. 1994; Brooks et al. 2002). Some researchers argue that stocking fry or small fingerlings is a more cost-effective solution because they are less expensive and more readily available (Festa et al. 1987; Reed and Staples 2017). Thus, a current debate in Walleye management is whether fry or fingerling stocked Walleye have improved survival and recruitment.

A major pitfall of many stocking programs is high levels of post-stocking mortality. Mortality rates during early life stages can surpass 95%, and small changes in survival rates can result in substantial differences in year-class strength (Houde 1997). Several biotic and abiotic factors are hypothesized to influence the success of Walleye stocking programs, including size at stocking (Santucci and Wahl 1993; Kampa and Hatzenbeler 2009), predation (Kim and DeVries 2001; Santucci and Wahl 1993; Johnson et al. 2015), prey availability (Ney and Orth 1986), competition (Raborn et al. 2004), winter severity (Pratt and Fox 2002), and variation in water temperature during and after stocking as well as seasonal trends (Clapp and Wahl 1997; Quist et al. 2003; Santucci and Wahl 1993; Hansen et al. 2015). The objective of this chapter is to review peer-reviewed literature assessing biotic and abiotic factors hypothesized to affect post-stocking survival, and recruitment of Walleye stocked at different life stages (e.g., fry and fingerling).

Biotic Factors

When Walleye are stocked, they are immersed into the middle of an established complex food web. Organisms at lower (e.g., prey), upper (e.g., predators), and adjacent (e.g., competitors) trophic positions all have the potential to directly and indirectly influence newly introduced Walleye. Each stocked system has unique food web interactions, making it challenging to assess which biotic interactions have the most significant effect on post-stocking survival. In addition to species interactions, body size plays a critical role in determining predators, competitors, and prey.

Size at stocking

Factors affecting growth and survival of fish changes during the first year of life and are often dependent on size (Santucci and Wahl 1993). The growth-mortality hypothesis (Biro et al. 2006; Stamps 2007) predicts that faster-growing individuals generally have higher survival rates due to "bigger-is-better" (Miller et al. 1988; Litvak and Leggett 1992; Meekan et al. 2006), "stage-duration" (Luecke et al. 1990; Houde 1997), and "growth-rate" (Anderson 1988) mechanisms. According to the "bigger-is-better" argument, larger individuals have (1) decreased susceptibility to gape-limited predators (Nilsson and Brönmark 2000), (2) access to a greater range of food types (Juanes and Conover 1994), and (3) greater competitive ability (Fausch and White 1981). Yet, several studies have failed to find selection favoring larger individuals (e.g., Quinn and Buck 2001; Sinclair et al. 2002; Carlson et al. 2004), maintaining interest in whether larger fish possess a survival advantage.

Numerous studies have attempted to evaluate the importance of size at stocking in relation to post-stocking survival of Walleye fingerlings. However, results from these studies have not provided a single recommendation that applies to all systems. For example, Santucci

and Wahl (1993) reported that fingerlings stocked at larger sizes (186-216 mm) had higher survival rates than those stocked at small (48-61 mm) and medium sizes (132-145 mm); however, these survival estimates were based on fall population estimates done one and two years after stocking. Similarly, Grote et al. (2018) reported size-selective mortality for smaller age-0 Walleye (100-220 mm) in three of four study lakes. Alternatively, Brooks et al. (2002) and Koppelman et al. (1992) reported that smaller fingerlings had higher survival rates than larger individuals. Lastly, Jennings and Philipp (1992), Olson et al. (2000), and Pratt and Fox (2002) reported that size was not a key factor influencing survival rates of Walleye fingerlings. Thus, there has been no "one size fits" all recommendation for the optimal size to stock Walleye.

A common theme among the research on Walleye stocking success is that size alone does not determine survival. For example, Brooks et al. (2002) noted large variability in relative survival rates of small (48-61 mm) and large Walleye fingerlings (95-110 mm) among systems and recommended that Walleye stocking should be based on lake-specific stocking assessments. Another hypothesis is that annual variability in survival influences the survival of differently sized Walleye. For example, in 1987, Jennings and Philipp (1992) found that Walleye fry survival was higher than small (50 mm) and large Walleye fingerling (100 mm), but in 1992, survival was greater for large fingerlings than smaller fingerlings, suggesting that annual variation plays a critical role in affecting survival (Jennings and Philipp 1992). Furthermore, Olson et al. (2000) hypothesized that predation and the ability to achieve piscivory had a greater influence on survival than just size alone. Lastly, size differences typically result in different stocking dates which are also hypothesized to influence post-stocking survival, because of its relationship with body size (Madenjian et al. 1991), prey availability (Fielder 1992; McDonnell et al. 2014), and water temperature (Quist et al. 2002).

Predation

Predation rates vary with size, abundance, species composition, and distribution of piscivores as well as the size of the Walleye being stocked (Chevalier 1973; Madenjian et al. 1991; Santucci and Wahl 1993; Fayram et al. 2005; Hoxmeier et al. 2006). Since predation is partially dependent on the gape size of the predator, predators of fry stocked Walleye are different from those of fingerling Walleye (Brooking et al. 1995). However, due to short residence times of consumed fry Walleye in predator stomachs (10-12 hours; Schneider 1997) as well as the challenge of identifying consumed fry in the guts of predators, few studies have assessed the effects of predation on fry stocked Walleye.

Some researchers have assessed predation in a field setting (e.g., reservoirs and ponds) by indirectly correlating fry survival with the density potential predators, where the abundance of Bluegill *Lepomis macrochirus* (Hoxmeier et al. 2006), Alewives *Alosa pseudoharengus* (Schultz 1992), Bullheads *Ameiurus sp*. (Carlander et al. 1960), and Yellow Bass *Morone mississippiensis* (Carlander et al. 1960) were associated with decreased survival rates of stocked Walleye fry. Alternatively, attempts have been made to directly assess the effects of post-stocking predation of stocked Walleye fry in controlled laboratory experiments (e.g., Alewives; Brooking et al. 1998). Like field studies, Alewives consumed a significant portion of Walleye fry stocked into observation tanks. Brooking et al. (1998) noted size could play a critical role on predation rates of Alewives. Adult Alewives (122 ± 1.52 mm total length) preyed intensely on larval Walleyes from the time larvae hatched until they reached about 16 mm total length; at 16-19 mm total length, larval Walleye started exhibiting avoidance behaviors and prolonged survival (Brooking et al. 1998). However, the threat of predation is not limited to the fry life stage with predation negatively influencing fingerling Walleye.

For post-stocked Walleye fingerlings, there is a short period of time (roughly 24-48 hours) after stocking when predation is thought to have substantial effects on survival (Freedman et al. 2012). Largemouth Bass *Micropterus salmoides* (Santucci and Wahl 1993; Fayram et al. 2005), Smallmouth Bass *Micropterus dolomieu* (Johnson and Hale 1977; Zimmerman 1999), Northern Pike *Esox lucius* (Anthony and Jorgensen 1977; Nate et al. 2003), Muskellunge *Esox masquinongy* (Bozek et al. 1999), and adult Walleye (Forney 1976) consume various sizes of Walleye fingerlings (Table 1.2). Several studies have provided recommendations on circumventing the negative effects of predation on recently stocked Walleye fingerlings. For example, in systems where Rainbow Smelt *Osmerus mordax* have significant effects on Walleye fingerlings survival, stocking Walleye fingerlings at sizes greater than the gape of Smelt minimizes predation (Lawson and Carpenter 2014). Alternatively, predator recognition training could decrease post-predation rates on Walleye fingerlings stocked into systems with large apex predators (e.g., Northern Pike, Largemouth Bass, and Muskellunge; Wisenden et al. 2004; Ferrari et al. 2010). A generality among predation research is to either stock Walleye too large for local piscivores as well as directly (i.e., put predators in rearing environment and all predators to consume some hatchery propagated fish) and indirectly (i.e., expose hatchery fish to predator scents in conjunction with alarm chemicals from injured conspecifics) exposing naïve hatchery fish to predators prior to stocking (Wisenden et al. 2004).

Prey

Food habits and food availability, as well as the ability to capture prey, are fundamental to juvenile fish growth and survival (Olson et al. 2000; Shoup and Wahl 2011). Like many other juvenile piscivores, Walleye undergo an energetically profitable ontogenetic diet shift during their first growing season (Stein et al. 2017). If recently stocked Walleye do not capitalize on

more energy profitable food sources (e.g., fish), a “bottleneck” may occur where growth is slowed or stopped due to the reliance on alternate lower-calorie prey items (e.g., zooplankton or invertebrates; Pelham et al. 2001). Thus, time of year, when stocking occurs dictates whether appropriate prey types and sizes are available at sufficient densities favorable for growth and survival (Knoll et al. 2011).

Walleye fry feed on rotifers, small copepods, and copepod nauplii (Smith and Moyle 1943; Houde 1967; Bulkey et al. 1976) and then switch to larger crustacean zooplankton such as cladocerans and *Daphnia sp.* (Mathais and Li 1982; Raisanen and Applegate 1983). Around 24 mm, Walleye start consuming larger prey items such as benthic macroinvertebrates (e.g., chironomid pupae and larvae; Bulkley et al. 1976) before shifting to piscivory around 50 mm (Forney 1966; Quist et al. 2002). Piscivorous fingerling Walleye are opportunistic predators that consume a variety of fishes (Table 1.1). However, at the piscivory stage, there is a limited window of time when fishes such as Yellow Perch *Perca flavescens*, Bluegill, and Gizzard Shad *Dorosoma cepedianum* are the appropriate size for consumption (Jackson et al. 1992; Pelham et al. 2001; Hoxmeier et al. 2006; Ward et al. 2008; Uphoff 2012; Uphoff et al. 2019). Thus, consideration of stocking time relative to prey availability has the potential to enhance post-stocking survival.

Competition

Competition can also affect the success of Walleye stocking programs (Raborn et al. 2004; Fayram et al. 2005). Gape size plays a critical role in determining diet composition and identification of potential competitors. Consequently, stocked Walleyes have a diverse array of potential competitors (e.g., planktivores, insectivores, and piscivores) due to ontogenic diet shifts.

Few studies have directly assessed competition between Walleye fry and other larval and planktivorous fishes. However, through qualitative observations and correlative assessment, some researchers have proposed that competitors of planktivorous Walleye include White Bass *Morone chrysops* (Rose 1955), Bluegill (Schneider 1997), and Gizzard Shad (Quist et al. 2004). Walleye fry potentially compete for copepods with a suite of age-0 fishes in natural systems, and competition could result in increased size differences between Walleye fry and their competitors.

In contrast to fry (total length = 9 mm), more information is available investigating potential competitive interactions for fingerling Walleye (total length: 98-300 mm). Competitors of fingerling Walleye include juvenile Smallmouth Bass (Wuellner et al. 2011), Largemouth Bass (Kelling et al. 2016), White Bass (Beck et al. 1998), age-1 Walleye (Parsons 1971), Saugeye *Stizostedion vitreum x S. canadense* (Bellgraph et al. 2008), and juvenile Northern Pike (Fayram et al. 2005). For example, diet overlap among age-0 and age-1 Walleyes in the summer occurs when age-0 Walleye shift from consuming invertebrates to fish (Parsons 1971). Similarly, competition between Gizzard Shad and small fingerling Walleye for zooplankton in Tuttle Creek Reservoir, Kansas, was hypothesized to affect survival rates of Walleye stocked at smaller sizes (Cramer and Marzolf 1970). Additionally, diet overlap was observed between Largemouth Bass and age-0 Walleye during August and September, which corresponds to stocking periods for larger fingerling Walleye (Kelling et al. 2016). During winter months, diet overlap has been observed between Walleye fingerlings and Striped Bass *Morone saxatilis* when they both consumed Gizzard Shad (Raborn et al. 2004). Like research evaluating the importance of prey species, competition can be dependent on temporal trends of prey availability and the size of Walleye and their competitors.

Abiotic Factors

Abiotic factors often have a significant effect on growth and survival of age-0 Walleye (Claramunt and Wahl 2000). Walleye researchers have focused much of their attention on evaluating the influence of winter severity and duration, as well as water temperature changes associated with stocking and seasonality. However, a challenge faced by many researchers is the inability to separate the influence of size from the aforementioned abiotic factors.

Winter severity

Winter severity and duration negatively affects overwinter survival of fishes (Fullerton et al. 2000; Murphy et al. 2006) due to reductions or cessation in feeding which results in slow growth (Conover 1992), energy depletion (Höök and Pothoven 2009), and poor energetic condition (Pratt and Fox 2002; Höök and Pothoven 2009). Reductions in feeding are especially critical for smaller individuals who have lower absolute energy reserves and higher weight specific metabolic rates than larger individuals (Sutton and Ney 2001). The first winter is the stage at which fishes are most susceptible to starvation (Shuter and Post 1990) as well as size-selective predation due to decreased growth rates (Grote et al. 2018). During winter months, smaller fingerlings have increased instantaneous rates of energy change (IEC per day) and length while larger fingerlings experienced a loss of instantaneous rates of energy change (IEC per day; Pratt and Fox 2002). Currently, it is unclear if size plays a critical role in overwinter survival of Walleye fingerlings (Kelso 1972; Forney 1977; Larscheid 1995), because there are instances where overwinter survival of Walleye fingerlings was not found to be a size selective process (Copeland and Carline 1998; Jonas and Wahl 1998; Pratt and Fox 2002).

Water temperature

Water temperature plays a crucial role in determining the success or failure of Walleye stocking programs both during and after the stocking process. Water temperatures on stocking day can have immediate effects on survival rates of Walleye (Quist et al. 2003). Stocked Walleye that experience sudden temperature changes had lower survival rates (Huh et al. 1976; Clapp et al. 1997; Harmon 2009). Walleye fry and fingerlings (48-61 mm) that experience temperature changes of at least 10°C had high mortality rates (20-22% 24 hours post-stocking; Santucci and Wahl 1993). Additionally, water temperatures following stocking events can influence survival by influencing growth rates. A challenge of assessing the relationship among thermal stress, mortality, and size of Walleye at stocking is that larger fingerlings are generally stocked in the fall when water temperatures are lower and less variable compared to the spring when fry are stocked, and temperature fluctuations are more common (Santucci and Wahl 1993). After stocking, changes in water temperature can influence Walleye growth (Kitchell et al. 1977; Uphoff et al. 2013) by altering metabolism (Higley et al. 1986) and food consumption rates (Johnson and Mathias 1994). Smaller body size and decreased growth rates during winter months can increase stocked Walleye vulnerability to predation (Grote et al. 2018), making stocked Walleye more susceptible to starvation and/or predation. However, during late spring increases in surface water temperature (e.g., 15°C) have been correlated closely with increases in zooplankton biomass, which influences survival of spring stocked fry and summer stocked small fingerling Walleye (36 mm total length; Fielder 1992). Additionally, water temperatures from May through June are reported to be positively related to growth of Walleye fingerlings (94-168 mm mean length) due to increases in abundances of prey (Serns 1982). Thus, water temperature plays a critical role in directly and indirectly influencing the survival of Walleye stocked.

Dissertation overarching objective

Due to the complexities of aquatic ecosystems, abiotic and biotic factors, directly and indirectly, influence survival of stocked Walleye (Hoxmeier et al. 2006), but which factors are important can vary widely among systems due to local environmental conditions and size of fish stocked. A wise recommendation from Ellison and Franzin (1992) was that "…Walleye stocking should be tailored to each system, not based on a set number and size of fish…" In reviewing the published literature, it is clear that every Walleye stocking event is unique, and that a challenge Walleye researchers and managers face includes distilling which biotic and abiotic factor is critical for enhancing survival, growth, and recruitment of stocked Walleye. With the improvements in rearing techniques and promotion of the concept "bigger-is-better," fingerling Walleye are being stocked at progressively larger advanced sizes (total length 100-300 mm) which provides the opportunity to evaluate whether abiotic and biotic factors that influence smaller stocked Walleye have the same effect on larger stocked individuals. Thus, the overarching objective of this dissertation is to evaluate the effects of abiotic (e.g., transportation practices, total length and condition at time of stocking, water temperature, stocked cohort) and biotic factors (e.g., predation and diet shifts) on stocked advanced fingerling Walleye survival (100-300 mm). In Chapter 2, I to assess changes in water chemistry and Walleye glucose, cortisol, and survival following transport to six lakes located between 16 and 487 km from the hatchery. In Chapter 3, I evaluate the relationships between transport duration, water quality, Walleye whole blood glucose, and plasma cortisol concentrations, and Walleye mortality using an experimental design that eliminated as many confounding factors as possible. In Chapter 4, I evaluate size-selective predation by Largemouth Bass, Smallmouth Bass, Northern Pike, Muskellunge, and adult Walleye on fall stocked age-0 Walleye. Specifically, I assessed whether

consumed age-0 Walleye total length was related to predator total length, predator gape height, and the probability of predation. Second, I compared length-frequency distributions of stocked Walleye to distributions of Walleye recaptured during fall sampling and Walleye recovered from the stomachs of predators to assess whether predators were consuming sizes of Walleye that made up larger proportions of the stocked population. In Chapter 5, I estimated post-stocking predation on age-0 Walleye (78-295 mm; 12-253 g) by a suit of predators (e.g., Largemouth Bass, Northern Pike, and adult Walleye). Specifically, I was interested in determining what percentage of stocked Walleye were consumed by predators, size-specific consumption of stocked Walleye, and temporal trends in consumption rates across predators. In Chapter 6, I compare fall diets of age-0 wild and stocked Walleye by evaluating the proportion of empty stomachs as well as proportions of zooplankton, benthic invertebrates, and fish in diets. In Chapter 7, I used mark-recapture modeling to estimate post-stocking apparent weekly survival rates of Walleye stocked into two systems (East Okoboji and West Okoboji) during fall 2015, 2016, and 2017. Finally, Chapter 8 summarizes general conclusions drawn from each chapter as well as provides insight as to future research directions.

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Tables

Table 1.1 Food items found in various size age-0 Walleye stomachs. For each prey item, there is reference to a specific study, reported Walleye length (mm), and system type (e.g., experimental lake, natural system in the field, or in a laboratory). n.d. indicated that this data was not included in the publication.

Common Name	Scientific Name/ Family	Walleye Length (mm)	System	Citation
Invertebrates				
Calanoid	*Copepoda*	36-59	Experimental Lake	Fox 1989
Chironomid	*Chironomidae*	36-59	Experimental Lake	Fox 1989
Chironomid	*Chironomidae*	36-59	Experimental Lake	Fox 1989
Cladocera	*Copepoda*	70-145	Natural System	Pelham et al. 2001
Copepoda	*Copepoda*	70-145	Natural System	Pelham et al. 2001
Daphnia	*Daphnia sp.*	36-59	Experimental Lake	Fox 1989
Diptera	*Diptera*	70-145	Natural System	Pelham et al. 2001
Gastropoda	*Gastropoda*	70-145	Natural System	Pelham et al. 2001
Fish				
Bluegill	*Lepomis macrochirus*	70-145	Natural System	Pelham et al. 2001
Bluegill	*Lepomis macrochirus*	100-210	Laboratory	Einfalt and Wahl 1997
Freshwater Drum	*Aplodinotus grunniens*	70-145	Natural System	Pelham et al. 2001
Freshwater Drum	*Aplodinotus grunniens*	n.d.	Natural System	Hartman and Margraf 1992
Gizzard Shad	*Dorosoma cepedianum*	100-210	Laboratory	Einfalt and Wahl 1997
Golden Shiner	*Notemigonus crysoleucas*	100-210	Laboratory	Einfalt and Wahl 1997
Iowa Darter	*Etheostoma exile*	70-145	Natural System	Pelham et al. 2001
Johnny Darter	*Etheostoma nigrum*	70-145	Natural System	Pelham et al. 2001
Logperch	*Percina caprodes*	70-145	Natural System	Pelham et al. 2001
Minnow *sp.*	*Clupeidae sp.*	Age-0	Natural System	Hartman and Margraf 1992
Rainbow Smelt	*Osmerus mordax*	Age-0	Natural System	Hartman and Margraf 1992
Spottail Shiner	*Notropis hudsonius*	70-145	Natural System	Pelham et al. 2001
Shiner *sp.*	*Cyprinidae*	Age-0	Natural System	Hartman and Margraf 1992
Temperate Bass	*Morone sp.*	Age-0	Natural System	Hartman and Margraf 1992
Walleye	*Sander vitreus*	70-145	Natural System	Pelham et al. 2001
Yellow Perch	*Perca flavescens*	n.d.	Natural System	Lyons and Magnuson 1987
Yellow Perch	*Perca flavescens*	n.d.	Natural System	Hartman and Margraf 1992

CHAPTER 2. EFFECTS OF TRANSPORT DURATION AND WATER QUALITY PARAMETERS ON AGE-0 WALLEYE *SANDER VITREUS* STRESS AND SURVIVAL

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Abstract

Age-0 Walleye *Sander vitreus* (hereafter referred to as Walleye) are stocked to achieve several management objectives. However, stocked Walleye are exposed to numerous handling and transport related stressors that can negatively influence disease resistance, survival, year-class strength, and fisheries management objectives. The objective of this study was to evaluate relationships between Walleye transport duration with changes in water chemistry parameters, Walleye whole blood glucose and plasma cortisol concentrations, and short-term (48 hr) mortality. Walleye were transported between 3.5 to 6 hours and stocked into holding cages located at one of six systems. Water quality and Walleye stress (via whole blood glucose and plasma cortisol) and mortality were evaluated prior to, during, and at 0, 2, 24, and 48 hours post-transportation. During transport, water temperatures generally decreased while carbon dioxide concentrations fluctuated between 2.7 and 22.5 mg/L. Walleye whole blood glucose and plasma cortisol concentrations varied by system and time since transport. Changes in carbon dioxide concentrations were associated with changes in whole blood glucose concentrations. However, cumulative survival rates and plasma cortisol concentrations were not associated with water quality parameters or transportation duration. Understanding Walleye tolerance to transportation

induced stress has the potential to enhance stocking programs by providing the opportunity for managers to make informed Walleye transportation practices.

Introduction

Walleye *Sander vitreus* is a highly valued sportfish in North America. In 2001, 3.8 million anglers spent approximately 51.9 million days angling for Walleye (USFWS-USCB 2002). The popularity of Walleye has resulted in situations where demand exceeds supply which has led to the development and implementation of stocking programs across the United States and Canada to establish new populations and supplement current populations (Barton 2011). In 2004, nearly 1.1 billion Walleye were stocked across the United States (Halverson 2008). However, Walleye stocking programs can have highly variable stocking success (Kerr 2007) that can result in weak year-classes (Willis and Stephen 1987; Johnson et al. 1996; Nate et al. 2000).

Two potential issues hindering stocking success are stress and mortality associated with transportation and handling procedures (Mitzner 1992). Numerous aspects of the transportation process influence physiological responses of Walleye that are indicative of stress. Prior research evaluating the effects of transportation practices on Walleye has focused on understanding physiological and behavioral responses associated with changes in water chemistry, handling, and transport duration. Walleye transported various durations (e.g.,1 to 5 hours) exhibit elevated physiological parameters (e.g., cortisol; Forsberg et al. 2001; Barton et al. 2003) immediately after transportation. In cases where Walleye were transported approximately the same duration but into different waterbodies, there was variability in elevated plasma cortisol concentrations, indicating that environmental conditions at the stocked waterbody may influence physiological responses (Barton et al. 2003). Additionally, Walleye transported approximately the same duration but into different waterbodies exhibited variable elevated plasma cortisol concentrations, indicating that environmental conditions among waterbodies may also influence

physiological responses (Fielder 1992; Clap et al. 1997; Barton et al. 2003). Understanding the relative importance of transport duration versus environmental conditions on stocked Walleye stress and survival could improve fish welfare and stocking success. However, there are many challenges associated with evaluating stress and mortality related to transportation of fish.

Despite prior Walleye transportation research, little information is available regarding the effects of transportation duration and discrepancies between the studies make it hard to compare results. Previous field evaluations on age-0 Walleye transported between one and three systems located at various distances reported changes in chloride, sodium, and cortisol concentrations as well as altered fish behavior (Forsberg et al. 2001; Barton et al. 2003). More recently, advances in fish production techniques now produce larger Walleye by their first fall (Summerfelt 2000; Brooks et al. 2002). Differences in Walleye size across transportation studies (see Forsberg et al. 2001; Barton et al. 2003) make it challenging to develop cross-study comparisons due to inverse relationships between metabolic rates and fish size, which influences changes in water chemistry parameters (e.g., dissolved oxygen and carbon dioxide) and physiological responses (e.g., glucose and cortisol; Brown et al. 1984; Yager and Summerfelt 1993). A majority of evaluations assess the effects of implementations to transport truck design and/or water treatment practices on transport truck water (see Wedemeyer 1996; Barton and Haukenes 1999; Forsberg et al 2001) with few studies simply observing changes in water chemistry during transport. In addition, not all studies consisted of evaluating hatchery-reared Walleye, which can have different physiological responses to handling relative to wild Walleye (Barton 2011). Thus, opportunities exist to improve our understanding of factors influencing age-0 Walleye transportation stress.

Our objectives were to assess changes in water chemistry and Walleye glucose, cortisol, and survival following transport to six lakes located between 16 and 487 km from the hatchery.

We hypothesized that Walleye transported longer durations will be exposed to greater changes in water chemistry during transport, will exhibit elevated whole blood glucose and plasma cortisol concentrations, and will have a lower probability of survival relative to Walleye transported for shorter durations. Collectively, our findings will provide insight on the effects of transportation duration and changes in water quality parameters on Walleye. Understanding the tolerance of Walleye to transportation has the potential to improve survival and recruitment of stocked Walleye.

Methods

Transportation protocol

This study took place during October 2015, 2016, and 2017 (Table 2.1). On each transportation date, a total of 1,550 age-0 Walleye (182 kg; 215 ± 28 mm; hereafter referred to as Walleye) were collected from a pond at Rathbun Fish Hatchery, Moravia, IA, USA and transported to one of six systems (i.e., individual lake on a specific stocking date) located between 16 and 487 km from the hatchery (Table 2.1). Walleye were transported to East Okoboji two times and are referred to as East Okoboji 15 and East Okoboji 17. Walleye were not fed in the 48 hours prior to transportation to decrease waste (e.g., ammonia and carbon dioxide) during transportation (Robb 2008). The transportation truck had three 1,260-L compartments that were all equipped with a ram-air ventilation system (Forsberg et al. 1999) and supplemental oxygen (0.2 to 0.4 liters per minute with a maintained tank pressure at 276 kPa). The rear tank was also equipped with OxyGuard Pacific water temperature probes that recorded water temperature and dissolved oxygen every 18 minutes. Approximately 514 Walleye (60.7 kg ± 0.25 kg) were transported in each tank during each event.

Edwards treatment

Once each compartment in the transportation truck was loaded, Edwards treatment was used to prevent transportation of Zebra Mussel *Dreissena polymorpha* veligers (Edwards and Babcock-Jackson 2002). Edwards treatment consisted of two steps. First, 750 mg/L of potassium chloride (muriate of potash; 98.8% KCL, Mosaic Global Sales, LLC, Lithia. FL) was added to each tank. After a one-hour exposure time, 25 mg/L of formalin (Parasite-S, Western Chemical, Ferndale, WA) was supplied to each tank for an additional two hours. In total, Edwards treatment took three hours to complete. Walleye transported to Rathbun Lake (16 km from hatchery) remained at the hatchery with each transport compartment lid open for three hours allowing for the completion of the Edwards treatment (Table 2.1). For all other systems, Edwards treatment commenced immediately after all transport truck tanks were loaded and the exposure times for each step occurred during the transportation process.

Post-transport monitoring

At each lake, Walleye from the rear tank were put into one of 12 cylindrical cages (2.4 x 2.4 x 1.2 m dimension; 40 Walleye per cage, 4.5 kg) that were anchored in 1.3 m of water parallel to shore. The remaining Walleye in the two other transport truck tanks were stocked into the system. The cages were buoyant and were vertically suspended in the water column just underneath the water surface. All cages were placed in locations void of plants with sediments ranging from cobble to sand. Walleye in six of the cages were used for the blood analysis. The other six cages were used to determine post-transport cumulative survival rates and for qualitative observations of lethargic behavior and lack of flight or fight responses. Walleye were held in cages for 48 hours post-transport. Blood analysis and mortality cages were placed in an alternating pattern. The cages were numbered from 1 to 12, and cages 1, 6, and 12 each had a

temperature HOBO pendant (HOBO Pendant® Temperature/ Light Data Logger, ONSET, Bourne, Massachusetts) that recorded water temperatures every 10 minutes.

Blood collection and analysis

At the hatchery, blood samples were drawn the morning of the experiment from 36 Walleye in the hatchery pond (pre-load) to establish a reference value (hereafter referred to as 'reference Walleye') and from 36 individuals after the entire transportation truck was loaded (post-load) to assess loading stress. Post-transportation blood samples were collected from 36 Walleye (six Walleye from each of the six blood analysis cages) immediately after all the cages were loaded (0 hour) and at 2, 24, and 48 hours after transportation. After a blood draw, each Walleye received a left pectoral fin clip to ensure that each individual only had blood drawn once. Clipping fins to identify Walleye does not influence mortality rates (Pratt and Fox 2002). Blood samples were collected using a 23-gauge needle with a vacutainer containing 143 units of sodium heparin. The needle was inserted ventrally adjacent to the anterior of the anal fin (Houston 1990) and 400 µL of whole blood was removed. Each vacutainer was inverted 10 times in order to mix the sodium heparin and the whole blood before being placed into an ice slurry. After all blood samples were collected, a sterile disposable pipette was used to remove 0.3 µL of whole blood that was placed directly onto a FreeStyle Lite test strip (FreeStyle Lite Meter, Abbott Diabetes Care, Inc., Alameda, California) to measure whole blood glucose concentrations (hereafter referred to as glucose; Ball and Weber 2017). The remaining sample was placed in a 14-mL centrifuge tube and spun at 3,500 rpm for 10 min to separate plasma from red blood cells (Gomes et al. 2005). A clean, disposable pipette was used to remove plasma from the top of the sample that was then placed in a microfuge tube and frozen at -80°C for future plasma cortisol processing in the laboratory at Iowa State University.

Plasma cortisol (hereafter referred to as cortisol) concentrations were determined via Cortisol ELISA Kit (Enzo Life Sciences Inc, Farmingdale, NY) which is a competitive immunoassay for the quantitative determination of cortisol in biological fluids. The kit uses a monoclonal antibody that binds to cortisol in a competitive manner. A standard curve was developed in conjunction with the enzymatic sample reactions. After an incubation time of two hours, the binding reaction was stopped, and the yellow color generated was read on a microplate reader at 405 nm. Cortisol concentrations were then based off the developed standard curve (Chard 1990).

Statistical analysis

Candidate mixed linear models were developed to evaluate relationships between whole blood glucose, plasma cortisol, and cumulative survival rates relative to water quality parameters associated with transportation (e.g., carbon dioxide, dissolved oxygen and changes in water temperature) and transportation duration. Due to the multiple observations over time during a 'transportation event' (i.e., each lake-year combination that was evaluated), it was of interest to incorporate a repeated measures correlation structure in the models. Candidate models were initially fit with both AR(1) and compound symmetric correlation structures. Models with the compound symmetric correlation structure had lower Akaike Information Criterion (AIC) values and were therefore used for the candidate models consisting of one of three response variables (i.e., whole blood glucose concentrations, plasma cortisol concentrations, or cumulative survival rates), time since transport (i.e., 0, 2, 24, and 48 hours), a single water quality or transport parameter (i.e., average transport truck dissolved oxygen, carbon dioxide, or water temperature, change in water temperature during stocking, or duration of transport), an interaction between time since transport and the water quality/transport parameter, and a random effect for the

'transportation event' to evaluate the relationship between physiological responses of the Walleye and water quality/transport parameters. An additional model for each response variable was also fit using maximum likelihood that excluded the water quality/transport parameter. The response variables (i.e., whole blood glucose, plasma cortisol, and cumulative survival) were averages of all Walleye samples at each time within a specific lake. Only two parameters (i.e., a water quality/transport parameter and time since transport) were included per model due to the lack of observations (<10) per predictor variable in the model (Peduzzi et al. 1995, 1996). Due to the low sample size, corrected Akaike Information Criterion (AIC_c) and corrected Akaike Information Criterion model weights were used to compare model performance across models with the same response variables. Data were analyzed using R 3.2.2 (Integrated development for R. R-Studio, Inc., Boston, USA).

Results

Water quality parameters

During transport, water temperatures ranged between 11°C and 21°C across all transportation events (Figure 2.1). Water temperatures in the transport truck generally declined 1.00°C (SD, 0.47°C). However, water temperature increased 2.59°C during transport to Blackhawk (Figure 2.1). Dissolved oxygen concentrations ranged between 9 and 12 mg/L (Figure 2.1). Dissolved oxygen concentrations remained around 10.68 mg/L (SD, 0.95 mg/L; Figure 2.1). Carbon dioxide concentrations ranged between 7 and 19 mg/L across all systems (Figure 2.1). Average lake water temperatures (± SD) around the cages at the time of stocking ranged between 5.09°C and 23.77°C and varied across systems (average temperatures calculated across the 3 HOBO pendants; Rathbun: 15.90 ± 0.05°C; Big Creek: 14.52 ± 0.19°C; Brushy Creek: 14.64 ± 0.19°C; Blackhawk: 15.41 ± 0.11°C; West Okoboji: 11.96 ± 0.34°C; East

Okoboji 15: 23.77 ± 0.19°C; East Okoboji 17: 5.09 ± 0.07°C). Walleye transported to Rathbun (4.88°C), East Okoboji 15 (12.22°C), and West Okoboji (2.73°C) experienced an increase in water temperature when stocked into the system while Walleye transported to Big Creek (-1.36°C), Blackhawk (-5.66°C), and East Okoboji 17 (-4.08°C) experienced a decrease in water temperature during stocking. Walleye stocked into Brushy Creek experience negligible water temperature changes during stocking.

Variation in glucose and cortisol concentrations

At 48 hours post-transport, Walleye stocked into Rathbun, Big Creek, Brushy Creek, Blackhawk, and West Okoboji had glucose and plasma cortisol concentrations that were either lower than or similar to pre-load concentrations (Figure 2.2A and B). Walleye transported to East Okoboji 15 and 17 had glucose and cortisol concentrations that remained elevated relative to pre-load concentrations (Figure 2.2A and B). Walleye transported to Big Creek, Brushy Creek, and West Okoboji had glucose concentrations that peaked at 2 hours post-transport, whereas Walleye transported to Rathbun had peak glucose concentrations immediately after being stocked into the lake and Walleye transported to East Okoboji 15 and 17 had glucose concentrations that peaked at 24 hours (Figure 2.2A). Walleye transported to Rathbun, Big Creek, Blackhawk, and West Okoboji had plasma cortisol concentrations that peaked at 2 hours since transport (Figure 2.2B). Walleye transported to East Okoboji 15 and 17 had cortisol concentrations that remained elevated at 24 hours since transport (Figure 2.2B).

Mixed linear models with plasma cortisol concentrations as a response variable had lower variance across transportation events relative to variation within a transportation event (Table 2.2). The candidate model most supported by AICc values with whole blood glucose as the response variable was the model that included transport truck carbon dioxide concentrations

(Table 2.2). There was a positive relationship between whole blood glucose concentrations and carbon dioxide concentrations at 0- and 2-hours post-transport; however, at 48 hours there was a negative relationship between carbon dioxide and whole blood glucose concentrations (Figure 2.3). The most supported candidate model with plasma cortisol as the response variable was the model that included the effects of time since transportation and transportation event but did not include transportation covariates (Table 2.2).

Survival rate

Immediately and 2 hours after transport, Walleye had survival rates close to 100% at all systems except for West Okoboji where survival 2 hours post-transport was lower (Figure 2.4). However, cumulative survival rates decreased across all systems 24 hours after transportation (Figure 2.4). At 48 hours since transport, Walleye transported longer durations (e.g., East Okoboji and West Okoboji) appeared to have lower cumulative survival rates than Walleye transported shorter durations (e.g., Rathbun, Big Creek, and Brushy Creek; Figure 2.4). Variance across transportation events were lower than variation within a transportation event across time (Table 2.2). For the models accounting for water quality variables, the model with the effects of time since transportation and transportation event but no transportation covariates was found to be the best fitting model based on the model evaluation metrics (Table 2.2).

Discussion

During transportation, we observed fluctuations in all water quality parameters (e.g., carbon dioxide, dissolved oxygen, and water temperature) as well as physiological blood parameters (e.g., whole blood glucose and plasma cortisol). This is similar to the findings in other Walleye transportation studies (*see* Forsberg et al. 1999; Forsberg et al. 2001; Barton et al. 2003). Glucose concentration was best explained by the model, including carbon dioxide

concentrations of the transport water, while cortisol concentrations and cumulative survival rates were best explained by models that did not include water quality parameters or transport duration. Overall, whole blood glucose, cortisol, and cumulative survival were not related to transport duration. Decreased cumulative survival after transportation in conjunction with potential increased susceptibility to predation and/ or starvation have the potential to negatively influence stocking success.

Typically, increased transportation duration is associated with increases in carbon dioxide concentrations (Nomura et al. 2009) and increases in water temperatures (Robertson et al. 1987; Forsberg et al. 1999). We observed that carbon dioxide concentrations varied during transport but did not approach concentrations associated with anesthetizing or euthanizing fish (150-250 mg/L carbon dioxide; Post 1979). The lack of a positive relationship between transport duration and carbon dioxide concentrations could be due to the type of circulation system on the transport truck as ram-air ventilation systems regulate carbon dioxide concentrations (Forsberg et al. 1999). However, glucose concentrations were influenced by carbon dioxide concentrations. There was a positive relationship between whole blood glucose and carbon dioxide concentrations at 0- and 2-hours post-transport but not at later time intervals, indicating the importance of monitoring carbon dioxide relative to the other water quality parameters we monitored and that elevated CO_2 likely only had an acute effect. During transport, we generally observed decreases in water temperatures. Variations in transport truck design (e.g., tank insulation, installations of heaters and/or chillers) influence how water temperature changes during transportation (Harmon 2009). Additionally, sudden changes in water temperature and quality elicit stress responses in fish, even when values are within the tolerance range of the species (Wedemeyer 1997) that can negatively influence survival (Harmon 2009). Walleye

exposed to temperature increases of 2-12°C can experience increased mortality related to effects of thermal stress (Clapp et al. 1997). Walleye transported to East Okoboji 15 (12.22°C) and West Okoboji (2.73°C) experienced increases in water temperature at time of stocking that could have influenced physiological responses, cumulative survival rates, and reflex impairment.

The magnitude of change and duration of time associated with elevated glucose and cortisol concentrations is a function of stressor type, intensity, and duration (Barton et al. 1986; Van Der Boon et al. 1991). Thus, Walleye transported for longer durations should elicit stress responses that are greater in magnitude relative to individuals transported shorter durations (Robertson et al. 1987; Mitzner 1992). However, whole blood glucose and plasma cortisol concentrations were better explained by models without transport duration. Similarly, duration of transport time was not positively correlated with physiological responses indicative of stress in trout *Oncorhynchus spp.*, but rather a response to initial stressors at the beginning of the stocking process (e.g., loading, netting, and handling; McDonald et al. 1993; Iversen et al. 1998). Likewise, Walleye transported to Rathbun and Big Creek had similar pre-load, 0, and 2 hours cortisol concentrations, indicating that holding Walleye for three hours in a sedentary transportation truck and transported 10 minutes causes a physiological response similar to individuals physically being transported for three hours. Similarly, handling and loading procedures can elicit a stress response similar to a short transport duration (0.5 hours; Ball and Weber i*n Review*). Thus, one strategy for minimizing transport related stress and mortality is to evaluate the initial handing procedures during the loading process (Miles et al. 1974; Johnson and Metcalf 1982; McDonald et al. 1993). Prolonged elevated concentrations of glucose and cortisol could make recently stocked Walleye more susceptible to the effects of other

environmental factors such as starvation (Santucci and Wahl 1993), predation (Freedman et al. 2012), and/ or disease (Bernoth and Crane 1995) and negatively influence stocking success.

Prolonged exposure to stressors (e.g., handling, transportation, and changes in water quality parameters) negatively influence glucose and cortisol concentrations that can ultimately result in mortality (Schreck et al. 2016). Mortality is a common problem when fish are transported from hatcheries or rearing ponds to stocking locations (Miles et al. 1974; Johnson and Metcalf 1982; Gomes et al. 2003; Pavlidis et al. 2003). Elevated physiological parameters of stress during transport can result in immediate mortality either during or after stocking or delayed mortality (Schreck et al. 2016). Alterations of reflex responses are whole animal indicators of a compromised physiological state and can be used as a predictor of delayed mortality (Davis 2010; Raby et al. 2012). During post-transportation observations, Walleye transported to East and West Okoboji appeared lethargic and demonstrated a lack of flight or fight response when cages were raised to count and remove dead Walleye. In contrast, Walleye transported shorter distances to Rathbun, Big Creek, Brushy Creek, and Blackhawk did not appear lethargic nor demonstrate a lack of flight or fight response. Similarly, Walleye transported six hours exhibited lethargic behaviors, but survival rates ten days post transport were high (99.8%; Forsberg et al. 1999). Additionally, we observed a decrease in cumulative survival rates with increasing transport duration. However, the survival candidate model that included transportation duration was not well supported.

Evaluating the effects of handling and transport stressors has the potential to improve stocking programs. However, evaluating the effects of transportation in the field is challenging due to confounding factors such as multiple transportation dates (Carmichael 1984; Forsberg et al. 2001), stocking fish into different waterbodies with variable water quality parameters (Barton,

Haukenes et al. 2003; Dobšíkova et al. 2006), and simultaneous changes of water quality parameters during transportation (e.g., pH, dissolved oxygen, carbon dioxide, and ammonia; Sampaio and Freire 2016). Similarly, in the current study, due to logistical constraints, several confounding factors limit our ability develop cross system comparisons. First, Walleye originated from several different hatchery rearing ponds. Variability in rearing environments across hatchery ponds could have resulted in variable Walleye pre-load glucose and cortisol concentrations among stocking events. Second, Walleye were not transported to all lakes on the same day that could explain the variable changes in water temperature during transport, which could have influenced physiological responses. Finally, stocking Walleye into different waterbodies with variable water quality parameters could have influenced post-transport physiological responses and mortality rates. We included a 'transportation event' variable in our model to account for variation among systems and stocking events while evaluating the potential effects of transportation condition variables (i.e., dissolved oxygen, carbon dioxide, water temperature, and transportation distance) on Walleye response metrics. Generally, across system variation was lower than within system variation, indicating that variation across transportation events should have had a lesser effect relative to variation associated with temporal changes seen within a transportation event. However, variation across systems may still have affected our ability to detect potential effects of transportation distance on Walleye stress and survival.

Stressors such as handling practices (e.g., seining, sorting, and netting) and variable transport duration have been associated with changes in water quality and fish glucose and cortisol concentrations (Iversen et al. 1998; Dobšíkova et al. 2006). Changes in water quality parameters and increased stress can lead to mortality in some instances (Bernoth and Crane 1995). We also observed changes in water quality parameters during the transportation process.

Changes in carbon dioxide concentrations were related with changes in glucose concentrations which are indicative of stress. Initially, we hypothesized that increased transportation duration would be associated with increased glucose and cortisol concentrations and reduced survival. However, whole blood glucose, cortisol, and cumulative survival rates were better explained by models not including transportation duration. Potentially, uncertainty associated with confounding factors (e.g., multiple sampling days and variability among Walleye in hatchery ponds) could have mitigated the effects of transportation duration. However, all transported Walleye had altered physiological parameters (e.g., glucose and cortisol concentrations) which could make recently stocked Walleye more susceptible to the effects of other environmental factors such as starvation, predation, and/or disease and therefore negatively influence post-stocking survival rates. Therefore, understanding these relationships will improve our understanding of the effects of transportation on fishes.

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Tables

Table 2.1 Identification of study systems, transportation date, driving distance from the Rathbun fish hatchery (km), and transport duration (hours).

System	Transportation Date	Driving Distance (km)	Transport Duration (hours)
Rathbun	October 26, 2015	16	3.50
Big Creek	October 19, 2015	169	3.75
Brushy Creek	October 21, 2016	275	4.75
Blackhawk	October 17, 2016	320	5.50
East Okoboji 15	October 29, 2015	480	6.00
East Okoboji 17	October 30, 2017	480	6.00
West Okoboji	October 31, 2016	487	6.15

Table 2.2 Linear mixed models with different response variables (i.e., average glucose, average cortisol, and cumulative survival) and various parameters associated with each stocking event; parameters assessed included average dissolved oxygen (DO; mg/L), carbon dioxide (CO_2; mg/L), and water temperature during transportation (TWT; °C) as well as change in water temperature during stocking (ΔSWT; °C) and transport duration (TD; hours). Each model included a for time since stocking effect (TST; hours), an interaction between the transportation parameter and time since stocking, and a random effect for transportation event (TE) effects. Variance within (error variance) and across (random effect variance) transportation events (± standard deviation), AIC_c, ΔAIC_c, and AIC_c weights were provided for each candidate model. Models are ranked according to their AIC_c values for each response variable.

Model Parameters	Across System Variance ± SD	Within System Variance ± SD	AICc	ΔAICc	AICc Weight
Glucose					
CO_2 + TST + (CO_2*TST) + TE	4024.00 ± 63.44	1163.00 ± 34.11	328.93	0.00	1.00
TST + TE	4900.00 ± 70.00	5424.00 ± 73.65	346.92	17.99	0.00
TWT + TST + (TWT*TST) + TE	2360.00 ± 48.58	3658.00 ±60.48	351.06	22.13	0.00
TD + TST + (TD*TST) + TE	5198.00 ± 72.10	4074.00 ± 63.83	357.81	28.88	0.00
DO + TST + (DO*TST) + TE	4332.00 ± 65.82	4665.00 ± 68.30	359.80	30.86	0.00
ΔSWT + TST + (ΔSWT*TST) + TE	4730.00 ± 68.77	5395.00 ± 73.45	363.55	34.62	0.00
Cortisol					
TST + TE	17.60 ± 4.20	83.08 ± 9.12	223.51	0.00	0.98
TD + TST + (TD*TST) + TE	21.37 ± 4.62	57.55 ± 7.59	232.25	8.74	0.01
CO_2 + TST + (CO_2*TST) + TE	0.67 ± 0.82	73.73 ± 8.59	233.06	9.55	0.00
ΔSWT + TST + (ΔSWT*TST) + TE	16.95 ± 4.12	71.82 ± 8.47	236.73	13.22	0.00
TWT + TST + (TWT*TST) + TE	18.78 ± 4. 33	74.68 ± 8.64	238.05	14.53	0.00
DO + TST + (DO*TST) + TE	15.89 ± 3.99	81.71 ± 9.04	239.72	16.21	0.00
Cumulative Survival					
TST + TE	20.48 ± 4.53	91.71 ± 9.58	226.45	0.00	0.96
CO2 + TST + (CO_2*TST) + TE	19.83 ± 4.45	63.35 ± 7.96	234.25	7.79	0.02
TD + TST + (TD*TST) + TE	13.41 ± 3.66	68.29 ± 8.26	234.72	8.27	0.02
DO + TST + (DO*TST) + TE	12.01 ± 3.47	86.34 ± 9.29	240.33	13.88	0.00
ΔSWT + TST + (ΔSWT*TST) + TE	20.37 ± 4.51	87.72 ± 9.37	242.28	15.83	0.00
TWT + TST + (TWT*TST) + TE	20.82 ± 4.56	90.18 ± 9.50	243.03	16.58	0.00

Figures

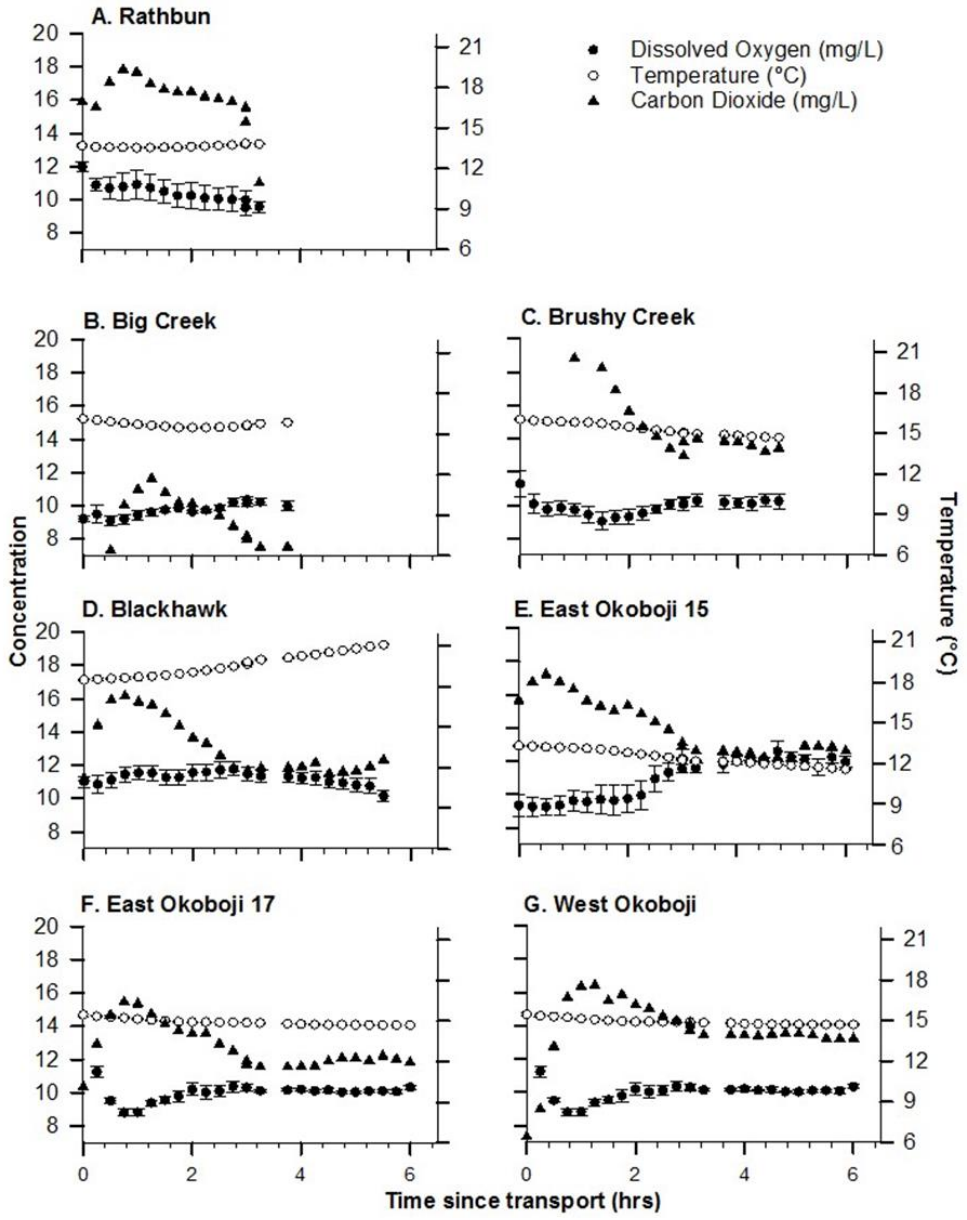

Figure 2.1 Comparison of average (± SE) dissolved oxygen concentrations (mg/L; solid circle) and water temperatures (°C; hollow circle) across three transport tanks and carbon dioxide (mg/L; solid triangle) concentrations in the rear transport tank during transportation to Rathbun (A), Big Creek (B), Brushy Creek (C), Blackhawk (D), East Okoboji (E & F), and West Okoboji (G). The values on the x-axis are relative to stocking time (e.g., 0 hrs).

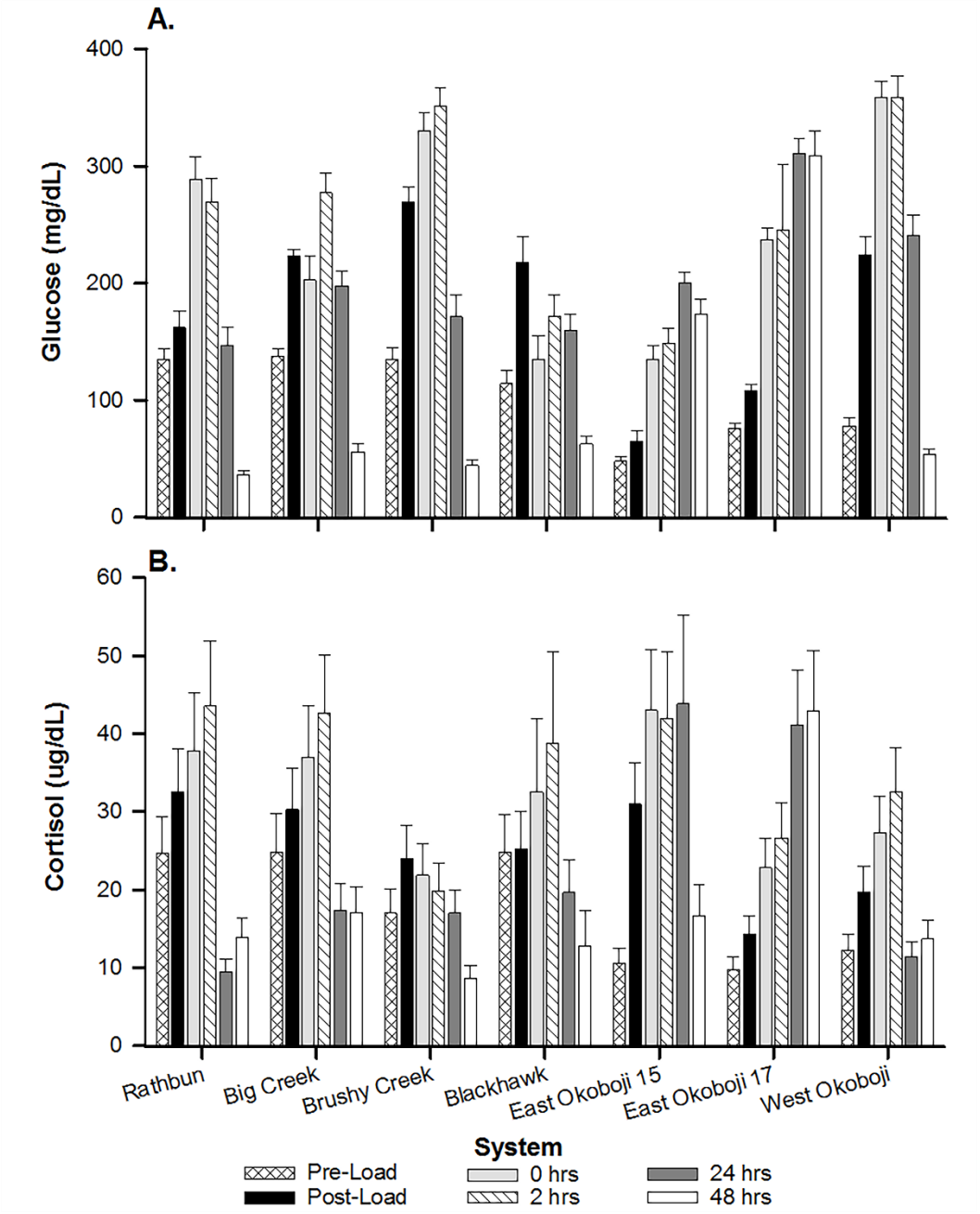

Figure 2.2 Comparison of temporal changes of average glucose (± SE; A) and cortisol (± SE; B) concentrations of Walleye transported different durations at different post- transport observation times (pre-load = white fill with black diamond pattern; post-load = black solid fill; 0 hours = light grey fill; 2 hours = white fill with horizontal black lines; 24 hours = dark grey fill; 48 hours = solid white fill).

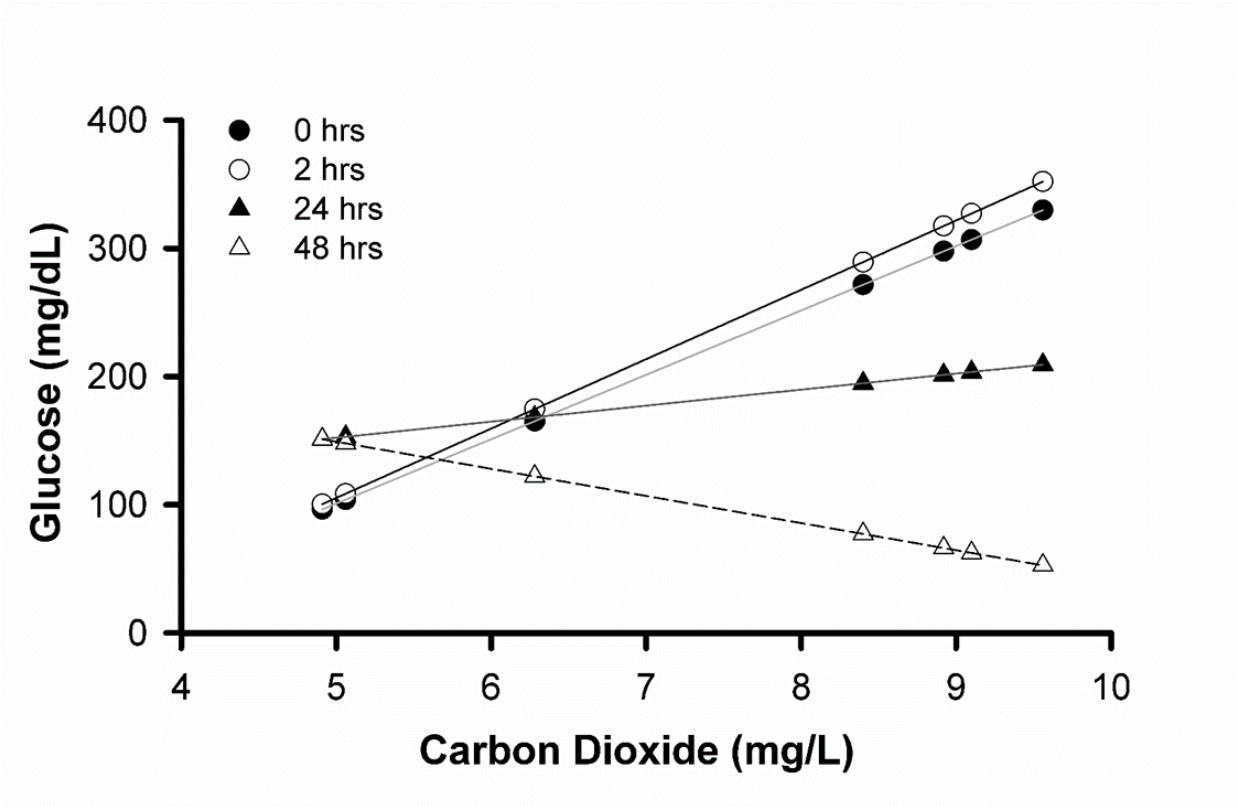

Figure 2.3 Comparison of whole blood glucose concentrations (mg/dL) relative to average carbon dioxide concentrations (mg/L) of transport truck water at 0 (solid circle), 2 (hollow circle), 24 (solid triangle), and 48 (hollow triangle) hours. Linear lines denoting marginal trends were developed for 0 (black), 2 (light grey), 24 (dark grey), and 48 (back and dashed line) hours. Output is based on the most supported whole blood glucose candidate model.

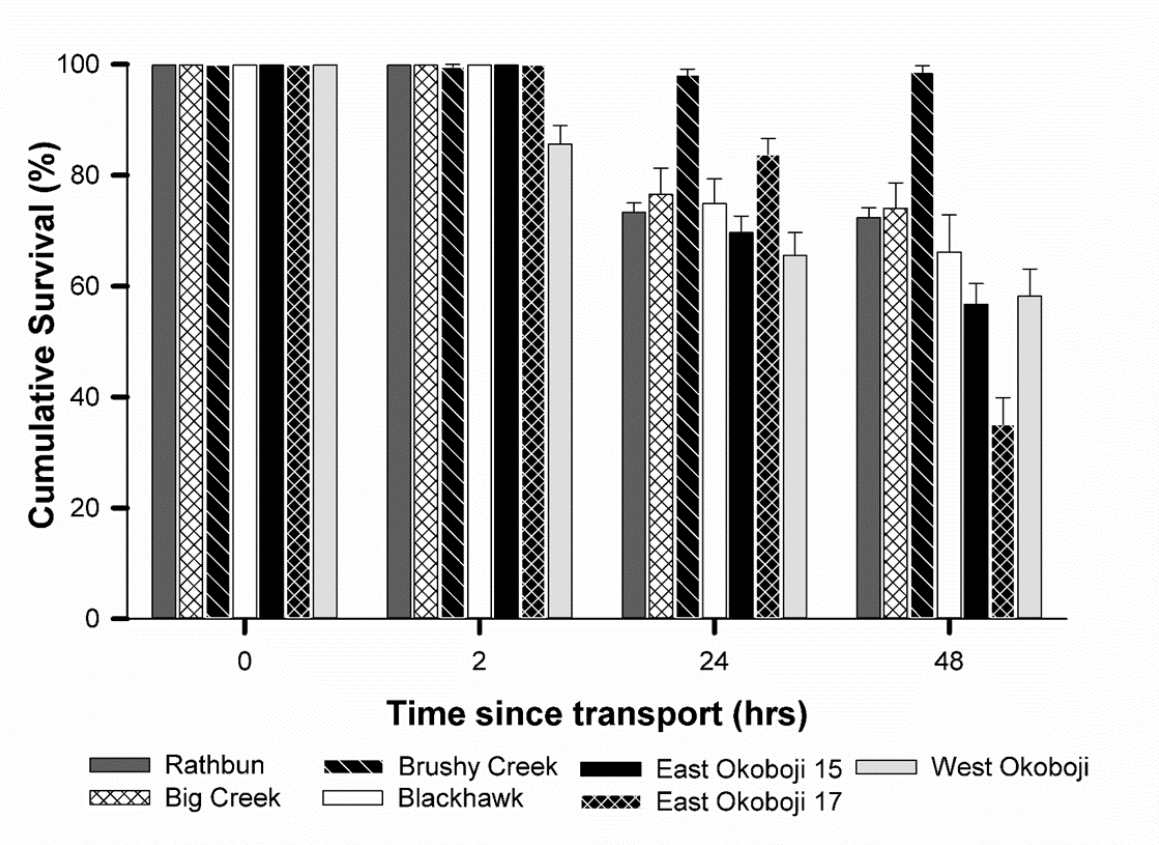

Figure 2.4 Average (± SE) cumulative survival rates (%) of Walleye transported to different systems (Rathbun = dark grey; Big Creek = white with black diamond pattern; Brushy Creek = black fill with white horizontal lines; Blackhawk = white; East Okoboji 15 = black; East Okoboji 17 = black fill with diamond pattern; West Okoboji = light grey) at 0, 2, 24, and 48 hours after transport. The systems increase in transport duration as you go from right to left within a transport event.

CHAPTER 3. RELATIONSHIPS AMONG TRANSPORT DURATION, WATER QUALITY, PHYSIOLOGICAL RESPONSES, AND MORTALITY RATES OF STOCKED AGE-0 WALLEYE *SANDER VITREUS*

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Abstract

Fisheries management agencies commonly stock Walleye *Sander vitreus* to establish new populations or enhance current populations. However, hatcheries are generally not located adjacent to stocking locations, and transportation duration can result in changes in water quality, physiology of transported fish (e.g., whole blood glucose and plasma cortisol), and reduced survival. Our objectives were to assess the effect of transport duration on water chemistry as well as changes in Walleye whole blood glucose and plasma cortisol concentrations and mortality. Walleye were transported 0, 0.5, 3, or 5 hours and stress and mortality were evaluated up to 48 hours post-transport. Total ammonia nitrogen concentrations, carbon dioxide, pH, and water temperature increased with transportation duration while total alkalinity of the transport water decreased. Plasma cortisol and whole blood glucose concentrations of Walleye transported longer durations took longer to return to baseline levels relative to those not transported. However, water quality parameters were not associated with changes in whole blood glucose and plasma cortisol concentrations. Despite increases in stress, mortality was low (2.5%) and similar among transportation durations. Information regarding transportation stress and mortality has the potential to improve the survival of stocked Walleye, which supports fisheries managers in achieving stocking management objectives.

Introduction

Fisheries managers stock hatchery-reared fish for a multitude of purposes, such as human consumption (put-take; Naylor et al. 2000), supplemental stocking programs aimed at enhancing current fish populations (Wedemeyer 2002), and establishment of new fish populations (Wedemeyer 2002). However, an obstacle of stocking hatchery-reared fish is unavoidable exposure to numerous handling and transport-related stressors (e.g., changes in water chemistry, transport duration, and crowding) that negatively influence health and survival, which can limit stocking success (Huntingford et al. 2006). Thus, evaluating the effects of handling and transport stressors on physiological changes can improve post-stocking survival. However, assessing the influence of aquaculture transportation and handling practices on physiological stress indicators (e.g., glucose and cortisol) in the field is challenging due to exposure to simultaneous transportation-related stressors and species-specific physiological responses (Barton 2000). Thus, species-specific evaluations are necessary.

There have been extensive experimental evaluations that have assessed various components of the transport process (e.g., handling, fish density, and changes in water quality) and their effects on fish physiology (e.g., glucose and cortisol concentrations) and changes in water quality, some of which include evaluations of different handling techniques, such as dry versus wet transfer (Wedemeyer 2002) and handling duration (Barton et al. 1985). Additionally, prior experimental evaluations have assessed the effects of the density of individuals in nets and holding containers and its association with increases in glucose and cortisol concentrations (Wedemeyer 1976; Gomes et al. 2003). Changes in water quality parameters, such as dissolved oxygen (Caldwell and Hinshaw 1994; Evans et al. 2003), ammonia (Randall and Tsui 2002; Wicks and Randall 2002), and carbon dioxide (Ross et al. 2001) can lead to increased glucose

and cortisol concentrations, and in some instances, mortality. However, to understand the effects of transportation practices on fish, simultaneous monitoring of changes in water chemistry, fish physiology, and handling techniques are necessary (Barton et al. 1985; Evans et al. 2003; Gomes et al. 2003). Therefore, developing an experimental methodology for simultaneously evaluating multiple components related to transportation has the potential to further our understanding of observations made in field evaluations regarding the behavior, health, and physiology of transported fish.

One of the most stressful periods for hatchery fish is during the transportation process. Field evaluations assessing the effects of transportation practices with physiological changes and mortality rates has received considerable attention (Urbinati et al. 2004; Lima and Oliveira 2018; Ball et al. 2020). Transportation duration has been associated with increased metamyelocytes and neutrophils of Common Carp *Cyprinus carpio* (Dobšíkova et al. 2006) as well as glucose concentrations of Largemouth Bass *Micropterus salmoides* (Carmichael 1984). A meta-analysis of published literature indicated a negative relationship between transportation duration and changes in glucose and cortisol (Sampaio and Freire 2016). However, challenges associated with field evaluations is unavoidable inclusion of confounding factors, such as multiple transportation dates (Carmichael 1984; Forsberg et al. 2001; Ball et al. 2020), stocking fish into different water bodies with variable water quality parameters (Barton et al. 2003; Dobšíkova et al. 2006), and simultaneous changes of water quality parameters during transportation (e.g., pH, dissolved oxygen, carbon dioxide, and ammonia; Sampaio and Freire 2016) can confound results. Developing an experimental design that eliminates as many confounding factors as possible (e.g., changes in water quality parameters, stocking water characteristics, and multiple

transportation days) has the potential to provide a more transparent evaluation of the effects of transportation duration on stress-related physiological changes and post-transportation mortality.

Walleye *Sander vitreus* is a commonly cultured sport fish in the United States (Lutz 1995; Barton 2011). Walleye stocking success is highly variable spatially and temporally but can often result in weak year-classes (Forney 1976). One potential factor influencing stocking success is Walleye stress and mortality associated with transportation and stocking (Mitzner 1992). For instance, age-0 Walleye (150-299 mm) transported between five to six hours exhibited lethargic behaviors with elevated plasma cortisol concentrations relative to individuals transported shorter distances (20-360 min; Forsberg et al. 2001), suggesting transportation duration elicits physiological and behavioral changes. However, the relationship between transportation duration and changes in physiological parameters indicative of stress is unclear due to confounding factors, such as multiple transportation events across multiple days and systems (Carmichael 1984; Forsberg et al. 1999; Ball et al. 2020). Additionally, larger Walleye (175-299 mm) are now being cultured and may exhibit diminished physiological stress responses associated with transportation due to the negative relationship between metabolic rates and fish size, which could influence the magnitude of change in water quality parameters (Brown et al. 1984; Yager and Summerfelt 1993). Therefore, our objectives were to evaluate the relationships between transport duration, water quality, age-0 Walleye whole blood glucose, and plasma cortisol concentrations, and age-0 Walleye mortality. We hypothesize that increased transport duration will be associated with adverse changes in water quality parameters such as increases in water temperature, carbon dioxide, and total ammonia nitrogen as well as decreases in pH and total alkalinity. Additionally, we hypothesize that with increasing transport duration, there will be increases in whole blood glucose and plasma cortisol concentrations as well as increases in

post-transport mortality rates (up to 48 hours post-transport).We hypothesized that increases in carbon dioxide, water temperature during transportation, transport water pH, unionized ammonia, total ammonia nitrogen, and total alkalinity will be associated with increases in whole blood glucose and plasma cortisol concentrations. Finally, we hypothesize that decreases in dissolved oxygen would be associated with increases in whole blood glucose and plasma cortisol concentrations. Unlike prior research, our evaluation will remove confounding parameters common in field evaluations (multiple stocking days and systems) as well as evaluate the effects of water quality parameters and transport duration on whole blood glucose and plasma cortisol concentrations of age-0 Walleye (175-266 mm) larger than those used in prior transport evaluations (e.g., Barton and Iwama 1991; Forsberg et al. 2001; Barton et al. 2003). Collectively, these results provide insight into the effects of transportation duration on age-0 Walleye. Understanding the tolerance of age-0 Walleye to transportation induced stress has the potential to alter and improve transportation practices, which will positively influence Walleye management goals.

Methods

Transportation protocol

On November 14, 2017, a total of 1,268 age-0 Walleye (136 kg of Walleye; 175-266 mm; hereafter referred to as simply ‘Walleye’) were collected from a single pond (pond #1) at Rathbun Fish Hatchery, Monrovia, IA, were exposed to one of four transportation durations (0, 0.5, 3, or 5 hours), and stocked into a new hatchery pond (pond #2). Handling and sample collection from Walleye was done under the guidance and approval from Iowa State University Institutional Animal Care and Use Committee (Iowa State University IACUC 7-15-8051-I). Walleye were not fed 48 hours before transportation to decrease waste (e.g., ammonia and

carbon dioxide) during transportation. The transportation truck had three 1,260 L compartments that were all equipped with a ram-air ventilation (RAV) system (Forsberg et al. 1999), supplemental oxygen (0.2 to 0.4 liters per minute with a maintained tank pressure at 276 kPa), and OxyGuard Pacific water temperature probes that recorded water temperature and dissolved oxygen every 18 minutes.

Before loading Walleye onto the transportation truck, each of the three transportation truck tanks were filled with freshwater that originated from the hatchery's flow-through system that filters water from Rathbun Lake through a 10-micron filtration system. All tanks were initially filled with water to provide equal weight distribution in the truck, but the middle and front tanks were drained and re-filled with fresh water just before being loaded with Walleye so that the water quality in the transportation truck was similar to the conditions in the pond that they were removed from (pond #1). After the transportation truck was filled with water, pond #1 was partially seined, and Walleye remained in the seine until they were loaded onto the transportation truck (up to 5 hours). The partial seining of pond #1 was done to minimize the number of disturbances (e.g., multiple seining events). We did not concentrate the entire population of Walleye in pond #1 into the seine because of stress responses related to density (e.g., crowding; Gomes et al. 2003). Changes in plasma cortisol concentrations after exposure to multiple, sequential, or simultaneous stressors indicates the cumulative effects of the stressors. Thus, efforts were taken to decrease the number of stressors not typical of transportation, such as multiple seining events (Barton et al. 1986). A staggered loading protocol was done so that all Walleye were transported on the same truck on the same day and allowed us to keep fish densities in each of the three transportation truck compartments consistent throughout the experiment. Additionally, this approach allowed us to unload fish from each transportation truck

tank simultaneously, so they were exposed to the same post-transport conditions (Specker and Schreck 1980). The procedure for loading the transportation truck began with every Walleye being netted, pushed across a wet sort table, and put into a metal sieve that was connected to a scale. Walleye were then placed in one of three tanks on the transport truck that were transported different amounts of time (0.5, 3, or 5 hours) or held in the low-density seine for 5 hours (no transportation). The staggered loading protocol started with loading the rear tank with 33.7 kg of Walleye, and then the truck was driven for two hours. Next, the middle transportation tank was loaded with 33.7 kg of Walleye, and Walleye in the middle and rear tanks were driven for two and a half hours. Finally, the front transportation tank was loaded with 33.7 kg of Walleye and Walleye in the front, middle, and rear tanks were driven for half an hour. The amount of time it took to fill each of the three-transportation tanks with water and 33.7 kg Walleye was 15 minutes. The amount of time the transportation truck was not moving while Walleye were being loaded was similar to the amount of time transportation trucks stop when on long transportation trips (e.g., stops for refueling). Additionally, no blood samples were collected from Walleye in each transport tank immediately after each transport tank was loaded.

After transportation, the truck returned to the hatchery and all Walleye received a fin clip to identify different transportation durations (e.g., transported 5 hours = right pelvic fin; transported 3 hours = left pelvic fin; 0.5 hours transported right pectoral fin; not transported-on transportation truck = left pectoral fin) before being put into holding cages. Clipping fins to identify Walleye exposed to different treatments does not influence mortality rates (Pratt and Fox 2002). Combinations of Walleye from each of the four transportation durations (0, 0.5, 3, and 5 hours) were either put into one of four rectangular cages (1.2 m x 1.2 m x 2.4 m) or into one of the twelve-cylinder cages (0.9 m x 0.9 m x 1.2 m) that were submerged in a hatchery pond (pond

#2). The density of Walleye in both cage types was 52 individuals/m^3 (rectangular cage = 192 Walleye per cage; cylindrical cage = 40 Walleye per cage). Individuals in the rectangular cages were used for the blood analysis portion of the experiment with each cage being used for a different blood draw time (0, 2, 24, and 48 hours after transportation), limiting the number of post-transportation disturbances as well as ensuring that no Walleye had multiple blood draws. Cylindrical cages were used to monitor mortality rates and make 3-minute observation periods during the 0, 2, 24, and 48-hour intervals on apparent lethargic behavior, which was observed as a lack of flight or fight responses. Similar to field transportation evaluations (Carmichael 1984; Iversen et al. 1998; Forsberg et al. 2001; Ball et al. 2020), each transportation event was only done once due to logistical constraints.

Blood collection and analysis

Blood samples were drawn the morning of the experiment at 7:00 AM from 20 Walleye in pond #1 to establish a reference value for Walleye (hereafter referred to as 'reference Walleye'). Post-transportation blood samples from 20 Walleye per transportation duration were drawn after all the cages were loaded (0 hours) and 2, 24, and 48 hours after transportation. It took 15-20 seconds per Walleye to collect each blood sample and each blood draw time took 10 to 13 minutes to collect all blood samples. At each blood draw time, Walleye were removed from different rectangular cages which removed the potential effects of prior sample collections on Walleye stress and allowed us to ensure that no Walleye had its blood drawn more than one time. A 23-gauge needle with a vacutainer containing 143 units of sodium heparin was inserted ventrally adjacent to the anterior of the anal fin (Houston, 1990) and 400 µL of whole blood was removed. Each vacutainer was inverted ten times to mix the sodium heparin and the whole blood before being placed into an ice slurry. After all blood samples were collected, a sterile disposable

pipette was used to remove 0.3 µL of whole blood that was placed directly onto a FreeStyle Lite test strip (FreeStyle Lite Meter, Abbott Diabetes Care, Inc., Alameda, California) to measure whole blood glucose concentrations ranging from 0 to 500 mg/dL (Ball and Weber 2017). The remaining sample was placed in a 14-mL centrifuge tube and spun at 3,500 rpm for 10 min to separate plasma from red blood cells (Gomes et al. 2005). A clean disposable pipette was used to remove plasma from the top of the sample that was then placed in a microfuge tube and frozen at -80°C for future plasma cortisol processing in the laboratory at Iowa State University.

Plasma cortisol concentrations were determined via Cortisol ELISA Kit (Enzo Life Sciences Inc, Farmingdale, NY), which is a competitive immunoassay for the quantitative determination of plasma cortisol in biological fluids. The kit uses a monoclonal antibody, which binds to plasma cortisol in a competitive manner. A series of known cortisol concentrations ranging from 156 to 10,000 pg/mL (10,000, 5,000, 2,500, 1,250, 625, 313 and 156 pg/mL) were used to develop a standard curve. Standard curve concentrations were developed in conjunction with the samples collected from Walleye. After an incubation time of two hours, the binding reaction was stopped, and the yellow color generated was read on a microplate reader at 405 nm. Plasma cortisol concentrations were then based on the developed standard curve (Chard 1995).

Water quality parameters

Water quality parameters (dissolved oxygen, pH, water temperature, total ammonia nitrogen, and total alkalinity) were measured in pond #1 before the loading of each transportation tank and in each compartment prior to loading, and each time the truck returned to the hatchery. Additionally, water quality parameters were measured in pond #2 with the holding cages at 24 and 48 hours after transportation. Dissolved oxygen (mg/L), pH, and water temperature (°C) were quantified with a HACH Multi HQ 40d (HACH Company, Loveland, CO). Carbon dioxide

concentrations (mg/L) were quantified with a LaMotte Carbon Dioxide DRT Kit (LaMotte Company, Chestertown, Maryland). The LaMotte Carbon Dioxide DRT Kit uses a titration method where two drops of Phenolphthalein Indicator 1% were mixed with 20 mL of sample water. A series of 1 mL drops of carbon dioxide reagent B (4253DR) were added to the solution and gently swirled until a faint pink color was produced and persisted for 30 seconds. The amount of carbon dioxide reagent B used in the titration represented the carbon dioxide concentration in parts-per-million (mg/L). Total ammonia nitrogen (TAN: mg/L) concentrations were measured with HACH TNT 830 Plus test tubes (HACH Company, Loveland, Colorado) in a HACH DR2800 spectrophotometer (HACH Company, Loveland, Colorado). A 5 mL water sample was added to each HACH TNT 830 Plus test tube solution and inverted three times, followed by a 15-minute reaction time. After 15 minutes, the test tube was put into the HACH DR2800 spectrophotometer at 694 nm. Un-ionized ammonia (mg/L; UIA) concentrations were calculated as (Wedemeyer 2001):

$$UIA = (TAN) * \left(\frac{percent\ unionized\ ammonia\ at\ pH\ and\ temperature}{100}\right)$$

where TAN is total ammonia nitrogen (mg/L). Percent unionized ammonia concentrations at a given pH and water temperature were obtained from a standardized table (Emmerson et al. 1975); pH was the pH of the source water at the same time in which the TAN sample was collected, and temperature was the temperature of the source water at the same time in which the TAN sample was collected. Finally, total alkalinity (mg/L $CaCO_3$) was quantified by swirling one phenolphthalein powder pill (HACH Permachem Reagent 94299) with a 100 mL water sample followed by the addition of one bromcrestol green-methyl red powder pill (HACH Permachem Reagent 943-99). The solution was titrated with 1.6N sulfuric acid (HACH Reagent

14389-01). OxyGuard Pacific water temperature and dissolved oxygen concentrations from each transportation tank were recorded every 18 minutes in each of the three compartments.

Statistical analysis

We developed candidate mixed linear models to evaluate relationships between whole blood glucose and plasma cortisol concentrations relative to water quality parameters associated with transportation (carbon dioxide, dissolved oxygen, water temperature, pH, total ammonia nitrogen, unionized ammonia, and total alkalinity) and transportation duration. For Walleye transported from pond #1 to pond #2 (no transportation), water quality parameters were based on values in pond #1. Due to multiple sequential observations associated with a 'transportation event' (0, 0.5, 3, 5 hours transported), a repeated measures correlation structure was incorporated in the models. Candidate models were fit with compound symmetric correlation structures. Candidate models consisted of one of two response variables (whole blood glucose or plasma cortisol concentrations), time since transport (0, 2, 24, and 48 hours), a single water quality or transport parameter (average carbon dioxide, dissolved oxygen, water temperature, pH, ammonia, unionized ammonia, and total alkalinity, or duration of transport), an interaction between time since transport and the water quality/transport parameter, and a random effect for the 'transportation event' to evaluate the relationship between physiological responses of the Walleye and water quality/transport parameters. A random effect was included in the model to account for variation unique to each transport event, such as time of day or diel changes in water quality. An additional model for each response variable was also fit using maximum likelihood that excluded the water quality/transport parameter. Thus, the model only consisted of time since transport and transportation event. The response variables (whole blood glucose and plasma cortisol) were averages of all Walleye sampled at each time from each 'transportation event.'

Only two parameters (a water quality/transport parameter and time since transport) were included per model due to the lack of observations (<10) per predictor variable in the model (Peduzzi et al. 1996). Due to the low sample size, corrected Akaike Information Criterion (AIC_c) and corrected Akaike Information Criterion model weights were used to compare model performance across models with the same response variables. Data were analyzed using R 3.2.2 (Integrated development for R. R-Studio, Inc., Boston, USA).

Results

Water quality parameters

During and after transportation, Walleye were exposed to dissolved oxygen concentrations ranging from 10.2 mg/L to 13.6 mg/L and water temperatures between 7.9˚C to 9.5˚C (Figure 3.1). Dissolved oxygen concentrations in each of the three transportation tanks initially decreased following the loading of Walleye into each compartment, followed by an increase (Figure 3.1). Additionally, the rear (5-hour transport) and middle (3-hour transport) tanks experienced a secondary decline followed by an increase in dissolved oxygen concentrations during transportation (Figure 3.1). Water temperature in the transportation truck gradually increased during transport, with maximum observed water temperature increasing with transport duration (Figures 3.1, 3.2). However, the increase in water temperature was <1.5°C. Carbon dioxide concentrations in the ponds ranged between 1 mg/L to 3 mg/L before and after transportation, whereas carbon dioxide concentrations ranged between 5.5 mg/L and 6.8 mg/L in all three transport tanks during transportation (Figure 3.2). Similarly, pH in the ponds (6.99-7.91) was lower than in the transport tanks after 2 hours for transportation (9.1 to 9.5; Figure 3.2). Total ammonia nitrogen and unionized ammonia concentrations in both ponds were low, with levels ranging between 0.026 to 0.083 mg/L and <0.01 mg/L, respectively (Figure 3.2).

However, total ammonia nitrogen and unionized ammonia concentrations increased with transport duration (Figure 3.2). Total alkalinity concentrations in the ponds and transport tanks varied between 66.5 to 93.0 mg/L $CaCO_3$ and tended to increase with transportation duration (Figure 3.2).

Physiological parameters

All transported Walleye had whole blood glucose concentrations that were higher than reference Walleye at all post-stocking monitoring times (0, 2, 24, and 48 hours post-transportation); however, Walleye transported 3 hours had whole blood glucose concentrations that were lower than reference Walleye at 48 hours post-transport (Figure 3.3). At each post-transport monitoring period (0, 2, 24, and 48 hours), Walleye transported 0, 0.5, 3, and 5 hours had variable whole blood glucose concentrations (Figure 3.3). Specifically, at 0 hours post-transport, whole blood glucose concentrations of Walleye transported 3-hours were higher than those transported 0.5 and 5 hours (Figure 3.3). At 2 hours post-transport, whole blood glucose concentrations of Walleye transported 3 hours was higher than those transported 0, 0.5, and 5 hours (Figure 3.3). At the third post-transport monitoring time (24 hours post-transport), whole blood glucose concentrations of Walleye not transported (0 hours transport time) was less than those transported 0.5, 3, and 5 hours, but during the first, second and fourth post transport monitoring times (0, 2, and 48 hours post-transport), Walleye not transported had whole blood glucose concentrations similar to Walleye transported 0.5 and 5 hours (Figure 3.3). Additionally, at the fourth post-transport monitoring time (48 hours post-transport), Walleye transported 3 hours had whole blood glucose concentrations lower than those transported 0, 0.5, and 5 hours (Figure 3.3).

In addition to the variation of whole blood glucose concentrations within specific post-transport monitoring times (0, 2, 24, and 48 hours), Walleye transported different durations of time (0, 0.5, 3, or 5 hours) exhibited temporal changes in whole blood glucose concentrations. Specifically, all transported Walleye had whole blood glucose concentrations that increased immediately after transportation and then decreased after two hours since transportation (Figure 3.3). However, between the second and third post-transport monitoring times (between 2 and 24 hours post-transport), Walleye transported 0, 3, and 5 hours had whole blood glucose concentrations that continued to decrease whereas Walleye transported 0.5 hours had whole blood glucose concentrations that remained elevated (Figure 3.3). Between the third and fourth post-transport monitoring times (24 to 48 hours post-transport), whole blood glucose concentrations of Walleye transported 0.5, 3, and 5 hours continued to decline, whereas whole blood glucose concentrations of Walleye not transported remained unchanged (Figure 3.3). Overall, the mixed linear model most supported by AIC*c* values with whole blood glucose as the response variable was the model that did not include a water quality parameter or transport duration (Table 3.1). The most supported candidate model, had a delta AIC_c value of zero and an AIC_c model weight of one, indicating that whole blood glucose concentrations varied across the post-transport monitoring times (0, 2, 24, and 48 hours) and that changes were unique to individual transportation events. Other candidate models that included glucose as a response variable were at least 37.8 delta AIC_c units away from the top candidate model and received no AIC_c model weight (Table 3.1)

Plasma cortisol concentrations for all transport durations were higher than reference Walleye values during the first and second post-monitoring times (0 and 2 hours post-transport; Figure 3.3). However, during the third post-stocking monitoring time (24 hours post- transport),

Walleye not transported had plasma cortisol concentrations similar to reference Walleye concentrations (Figure 3.3). Similarly, during the fourth post-stocking monitoring time (48 hours post-transport), Walleye transported 3 hours had plasma cortisol concentrations similar to reference Walleye concentrations (Figure 3.3). At the first post-stocking monitoring time (0 hours post-transport), plasma cortisol concentrations of Walleye transported 3 hours were higher than those transported 0.5 and 5 hours (Figure 3.3). At the second post-stocking monitoring time (2 hours post-transport), plasma cortisol concentrations of Walleye transported 3 hours were higher than those transported 0, 0.5, and 5 hours (Figure 3.3). At the third post-stocking monitoring time (24 hours post-transport), plasma cortisol concentrations of Walleye transported 0 hours were less than those transported 0.5 and 5 hours (Figure 3.3). Lastly, during the fourth post-stocking monitoring time (48 hours post-transport), plasma cortisol concentrations of Walleye transported 3 hours were lower than Walleye transported 0, 0.5, and 5 hours (Figure 3.3).

Similar to whole blood glucose concentrations, plasma cortisol concentrations exhibited temporal changes. Plasma cortisol concentrations of Walleye transported 3 hours continually decreased after transport while Walleye transported 0.5 and 5 hours had plasma cortisol concentrations that initially decreased but then remained constant and Walleye not transported had plasma cortisol concentrations that increased (Figure 3.3). Mixed linear models with plasma cortisol as a response variable had lower variance across transportation events relative to the variation within a transportation event (Table 3.1). Similarly, the most supported whole blood glucose candidate model, the most supported mixed linear model with plasma cortisol as the response variable, did not include a water quality parameter for transport duration (Table 3.1). The most supported candidate model with plasma cortisol as the response variable, had a delta

AIC_c value of zero and an AIC_c model weight of one, indicating that plasma cortisol concentrations varied across the post-transport monitoring times (0, 2, 24, and 48 hours) and that changes were unique to individual transportation events. Other candidate models that included glucose as a response variable were at least 38.7 delta AIC_c units away from the top candidate model and received no AIC_c model weight (Table 3.1).

Mortality rate

During post-transportation observations, Walleye did not appear lethargic nor demonstrate a lack of flight or fight response when cages were raised to count and remove dead Walleye as well as make 3-minute behavior observations. Mortality was low throughout the 48 hours since transportation monitoring time, with only one mortality, a Walleye transported 0.5 hours, occurring 2 hours after transport (2.5% of that treatment).

Discussion

During transportation, we observed fluctuations in all water quality parameters (e.g., carbon dioxide, dissolved oxygen, water temperature, pH, ammonia, and total alkalinity) as well as Walleye physiological blood parameters (e.g., whole blood glucose and plasma cortisol), all of which are commonly reported in transportation studies (*see* Forsberg et al. 1999, 2001; Barton et al. 2003; Ball et al. 2020). During transportation, we observed increasing total ammonia nitrogen and total alkalinity concentrations. However, we did not note any positive or negative relationships between hauling duration and dissolved oxygen, water temperatures, carbon dioxide, and pH concentrations. Despite changes in water quality parameters, whole blood glucose and plasma cortisol concentrations were not associated with changes in water quality parameters. Whole blood glucose and plasma cortisol concentrations tended to increase with transport duration, with Walleye transported longer durations having whole blood glucose and

plasma cortisol concentrations that remained elevated for a longer duration. Furthermore, we observed low mortality rates (2.5%) and no lethargic behavior after transportation. Collectively, our results suggest that transportation of Walleye does elicit physiological changes indicative of stress; however, these physiological changes were not associated with various water quality parameters nor resulted in significant mortality.

Previous research on Walleye transportation focused on the effects of water chemistry, such as water salinity, buffering capacity, and carbon dioxide concentrations (Forsberg et al. 1999, 2001; Barton et al. 2003). We hypothesized that there would be a positive relationship between transport duration and water temperatures and concentrations of carbon dioxide and total ammonia nitrogen. We did find support for our hypotheses related to water temperature, carbon dioxide concentrations, and total ammonia nitrogen. Typically, increased transportation duration is associated with increased ammonia (Dobšíkova et al. 2006; Nomura et al. 2009), carbon dioxide concentrations (Nomura et al. 2009), and water temperatures (Forsberg et al. 1999) and decreases in pH (Dobšíkova et al. 2006). Dissolved oxygen and carbon dioxide concentrations in each transport truck tank were different from concentrations in pond #1; however, changes in these parameters were not associated with transport duration. Additionally, carbon dioxide concentrations in each transport tank did not approach levels associated with anesthetizing or euthanizing fish (150-250 mg/L carbon dioxide; Post 1979). When transporting fish, it is crucial to consider the type of circulation system because this can influence changes in water chemistry parameters. Ram-air ventilation systems, like the one on the transportation truck we used, regulate carbon dioxide concentrations (Forsberg et al. 1999). However, changes in water temperature, pH, ammonia, and alkalinity were affected to various degrees by transport duration. Total alkalinity, pH, water temperature, and total ammonia nitrogen concentrations are

interrelated and influence the percentage of total ammonia nitrogen that is in the unionized form (Summerfelt 2000). Thus, decreases in total alkalinity would allow pH to increase, which is associated with increases in the percentage of un-ionized ammonia (Summerfelt 2000). The highest observed un-ionized ammonia concentration during our study was 0.72 mg/L, which is near the 1.06 median lethal level for Walleye (Mayes et al. 1986). Therefore, Walleye transported five or more hours may be exposed to potentially lethal concentrations of un-ionized ammonia that likely affects Walleye stress and mortality. We also initially hypothesized that changes in water quality parameters would be associated with increased levels of whole blood glucose and plasma cortisol. However, there was little evidence supporting this hypothesis. Overall, changes in dissolved oxygen, carbon dioxide, ammonia, unionized ammonia, water temperature, pH, alkalinity, or total alkalinity were not associated with changes in whole blood glucose and plasma cortisol concentrations. Similarly, changes in water temperature, dissolved oxygen, and transport duration were not related to changes in plasma cortisol concentrations or cumulative survival rates of transported age-0 Walleye; however, changes in plasma glucose concentrations were related to carbon dioxide concentrations during transport (Ball et al. 2020). Changes in whole blood glucose and plasma cortisol could have been influenced by stressors associated with the physical handling of age-0 Walleye or some other stressor unique to each transportation event (i.e., the act of being in an uncrowded seine for various amounts of time, or multiple instances of hatchery personnel entering pond #1). Thus, similar to field evaluations, unavoidable confounding factors, such as being in an uncrowded seine for various amounts of time, or multiple instances of hatchery personnel entering a pond could have influenced some of our observations.

The magnitude of change and duration of time associated with whole blood glucose and plasma cortisol concentrations returning to reference levels is a function of stressor type, the number of stressors, and stressor duration (Barton et al. 1986). Thus, Walleye exposed to longer transportation durations, and a greater number of stressors (e.g., handling and transportation) should exhibit higher concentrations of whole blood glucose and plasma cortisol relative to individuals transported a shorter distance and those exposed to fewer stressors. We initially hypothesized a positive relationship between transport duration and concentrations of whole blood glucose and cortisol. However, we found little evidence supporting our hypothesis. Similarly, changes in whole blood glucose and plasma cortisol of Walleye transported between 3.5-6.15 hours were not strongly related to transport duration (Ball et al. 2020). Similarly, transport duration was not positively correlated with physiological responses indicative of stress in trout *Oncorhynchus spp.* (e.g., loading, netting, and handling; McDonald et al. 1993; Iversen et al. 1998). Potentially, the lack of relationship between transport duration and changes in whole blood glucose and plasma cortisol could partially be due to alterations in metabolic processes due to extended exposure to a stressor (e.g., transportation duration) that might allow for acclimation (Sampaio and Freire 2016). Thus, whole blood glucose and plasma cortisol concentrations of Walleye transported five hours could have decreased during transportation, which would explain why Walleye transported five hours had lower whole blood glucose and plasma cortisol concentrations than those transported three hours. The duration of time required for physiological parameters to return to reference concentrations (concentrations before handling and transport) is variable. For example, plasma cortisol concentrations of Eurasian Perch (Acerete et al. 2004), Rainbow Trout *Oncorhynchus mykiss*, Brook Trout *Salvelinus fontinalis*, and Lake Trout *Salvelinus namaycush* (Barton 2000) transported various durations (e.g., 4 and 2

hours) did not return to control concentrations within 48 hours post-transport while whole blood glucose and plasma cortisol concentrations of Tambaqui *Colossoma macropomum* transported 10 hours (Gomes et al. 2003) and Walleye transported 5 hours (Forsberg et al. 2001) returned to values similar to resting concentrations 24 hours after transportation. We observed that Walleye exposed to different transportation durations exhibited elevated plasma cortisol and whole blood glucose concentrations relative to reference pre-transportation concentration. However, Walleye not transported recovered from handling within 24 hours, whereas those transported either 0.5, 3, or 5 hours still had elevated whole blood glucose and plasma cortisol concentration. Exposing fish multiple stressors (e.g., handling and transportation) resulted in changes in whole blood glucose and plasma cortisol concentrations as well as concentrations that remain elevated for longer durations of time (Barton et al. 1986). Therefore, stocking programs should consider the number of stressors as well as stressor type and duration, because the cumulative effects of stressors have the potential to influence Walleye behavior, making them more susceptible to predation (Freedman et al. 2012) and starvation (Santucci and Wahl 1993). Increased post-stocking predation has the potential to remove a significant portion of stocked populations (Grausgruber and Weber, *in review*), which can negatively influence the success of stocking initiatives and management goals.

Exposure to multiple stressors produces a cumulative stress response that may be more than the sum of individual stressors and, in some instances, result in mortality (Power 1997). We initially hypothesized that with increased transportation there would also be an increase in mortality rates; however, our data did not support this hypothesis. We observed low mortality rates, with only one mortality occurring two hours after transportation. Similarly, Black Bass *Micropterus* spp. (Carmichael 1984), Matrinxã *Brycon cephalus* (Urbinati et al. 2004), and

Striped Bass *Morone saxatilis* (Mazik et al. 1991) all experience low mortality following transportation. However, Walleye transported five hours have been observed to exhibit lethargic behaviors but resulted in only a few mortalities (0.4-1.8%; Forsberg et al. 1999). Similarly, Walleye transported longer durations (6-6.15 hours) can exhibit a lack of fight-or-flight responses while Walleye transported shorter durations (<5 hours) exhibit no observable changes in fight-and-flight responses, but exhibit variable cumulative survival rates ranging from 0-60% 1-2 days post-stocking (Ball et al. 2020). Across study variability in mortality rates, could, in part, be due to confounding factors associated with field evaluations (i.e., variability associated with multiple stocking locations and dates; Forsberg et al. 1999; Ball et al. 2020). However, the lack of observed lethargic behavior and few mortalities in our study could partially be due to low water temperatures (8.0-9.5℃). Lower water temperatures can reduce physiological responses in fish, which can result in decreased mortality (Louison et al. 2017). Thus, transporting Walleye at low temperatures (8.0-9.5℃) could have a positive cascading effect on the metabolic rates of Walleye, which would influence changes in water quality, physiological responses, and mortality rates.

No matter the fisheries management stocking objective, the success of a stocking initiative is highly dependent on post-stocking survival. Prior research has noted that handling practices (e.g., seining, sorting, and netting) paired with variable transport duration have been associated with changes in water quality and physiological responses of fish (e.g., whole blood glucose and plasma cortisol concentrations; Iversen et al. 1998; Dobšíkova et al. 2006). Exposure to reduced water quality during transportation paired with increased stress can result in disease and, in some instances, increases in post-stocking mortality (Bernoth and Crane 1995; Murphy and Lewbart 1995). Similar to field evaluations, we observed that in a more controlled

environment, handling and transporting Walleye elicits physiological responses indicative of stress. Additionally, handling procedures such as netting and being moved across a sorting table was associated with physiological changes similar to Walleye that are transported, which suggests that handling procedures before transportation could play a significant role in physiological responses measured after transportation. However, changes in physiological parameters (whole blood glucose and plasma cortisol) were not related to transportation duration, or water quality parameters (dissolved oxygen, carbon dioxide, water temperature during transportation, transport water pH, unionized ammonia, total ammonia nitrogen, and total alkalinity). Overall, Walleye transported different durations had high survival. However, elevated concentrations of whole blood glucose and plasma cortisol could make recently stocked Walleye more susceptible to the effects of other environmental factors such as starvation, predation, and disease, all of which could result in increased mortality rates shortly after stocking events. Results from the current study suggest that unionized ammonia concentrations can reach near lethal concentrations, especially when Walleye are transported five hours. Thus, fisheries agencies stocking Walleye should consider monitoring changes in water quality and physiology to limit indirect adverse effects associated with stocking stressed individuals (e.g., increased post-stocking predations, disease, and starvation).

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Tables

Table 3.1 Linear mixed models with different Walleye physiological response variables (i.e., average whole blood glucose or average plasma cortisol) and various parameters associated with each stocking event; parameters assessed included average dissolved oxygen (DO; mg/L), carbon dioxide (CO_2; mg/L), water temperature during transportation (WT; °C), transport water pH (pH), unionized ammonia (UIA; mg/L), total ammonia nitrogen (A; mg/L), total alkalinity (TA; mg/L $CaCO_3$), and transport duration (TD; hours). Each model included time since stocking effect (TST; hours), an interaction between water quality parameter or transportation duration and time since stocking, and a random effect for each transportation event was included in the model to account for variation unique to each transport event, such as time of day or diel changes in water quality. Variance within (error variance) and across (random effect variance) transportation events (± standard deviation), AIC_c, ΔAIC_c, and AIC_c weights were provided for each candidate model. Models are ranked according to their AIC_c values for each response variable.

Model Parameters	Across TE Variance ± SD	Within TE Variance ± SD	AIC*c*	ΔAIC*c*	AIC*c* Weight
Glucose					
TST + TE	124.7 ± 11.17	1310.5 ± 36.20	182.88	0.00	1
DO + TST + (DO*TST) + TE	<0.001 ± <0.001	1047 ± 32.36	220.67	37.79	0
UIA + TST + (UIA *TST) + TE	<0.001 ± <0.001	798.9 ± 28.27	240.34	57.46	0
pH+ TST + (pH*TST) + TE	<0.001 ± <0.001	814.7 ± 28.54	240.65	57.77	0
A + TST + (A*TST) + TE	<0.001 ± <0.001	817.7 ± 28.59	240.71	57.83	0
TD+ TST + (TD*TST) + TE	<0.001 ± <0.001	841.8 ± 29.01	241.17	58.29	0
TA+ TST + (TA*TST) + TE	<0.001 ± <0.001	931.90 ± 30.53	242.80	59.92	0
WT+ TST + (WT*TST) + TE	<0.001 ± <0.001	944.7 ± 30.74	243.02	60.14	0
CO_2 + TST + (CO_2*TST) + TE	<0.001 ± < 0.001	1042 ± 32.29	244.60	61.71	0
Cortisol					
TST + TE	5213.00 ± 72.20	233348.00 ± 483.10	264.85	0.00	1
DO + TST + (DO*TST) + TE	3052.00 ± 55.25	183679.00 ± 428.58	303.60	38.75	0
WT+ TST + (WT*TST) + TE	<0.001 ± <0.001	139393.00 ± 373.40	322.93	58.08	0
TA+ TST + (TA*TST) + TE	<0.001 ± <0.001	140436.00 ± 374.7	323.05	58.20	0
CO_2 + TST + (CO_2*TST) + TE	<0.001 ± <0.001	155910.00 ± 394.90	324.72	59.87	0
UIA + TST + (UIA *TST) + TE	<0.001 ± <0.001	174900.00 ± 418.20	326.56	61.71	0
pH+ TST + (pH*TST) + TE	<0.001 ± <0.001	183514.00 ± 428.40	327.33	62.48	0
A + TST + (A*TST) + TE	374.60 ± 19.35	183678.90 ± 428.58	327.37	62.53	0
TD+ TST + (TD*TST) + TE	2691.00 ± 51.88	183679.00 ± 428.58	327.57	62.72	0

Figures

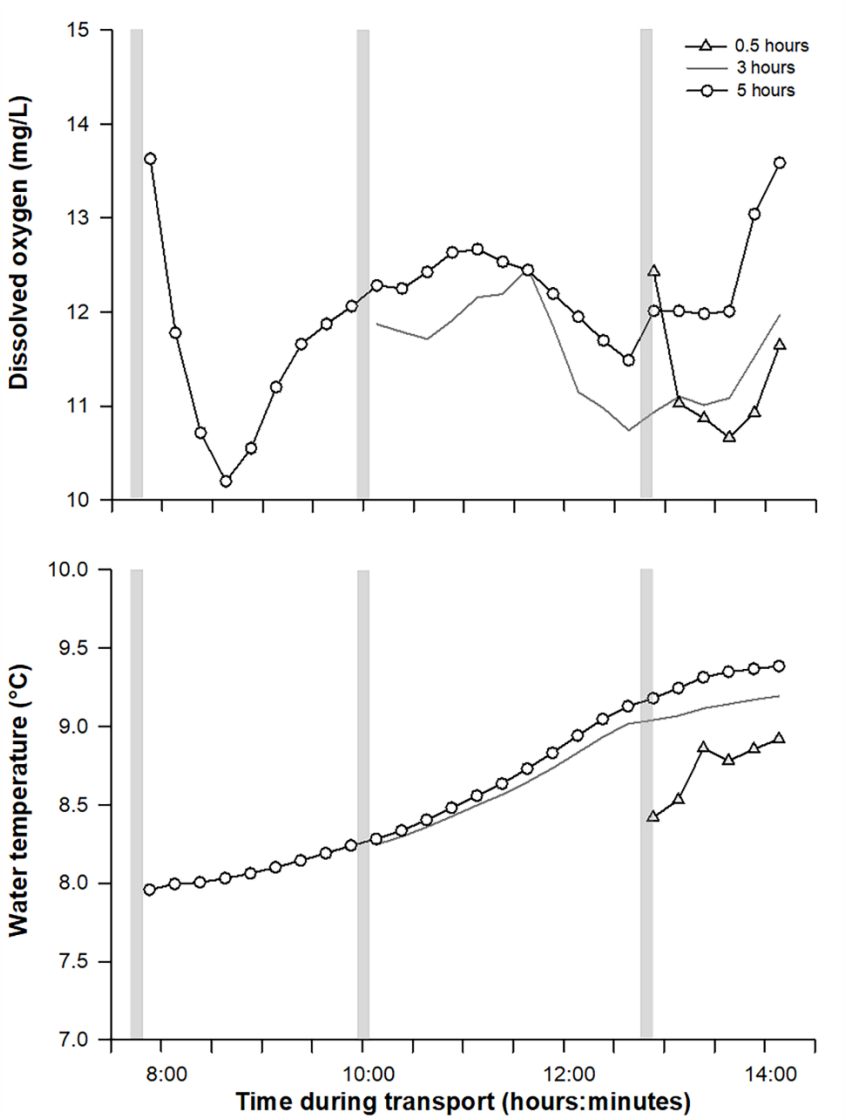

Figure 3.1 Changes in dissolved oxygen (mg/L) and water temperature (˚C) in transport treatments. Grey boxes denote times when the transportation truck was at the hatchery and being loaded with Walleye *Sander vitreus*. The line with circles denotes water parameters in the rear transportation tank which transported Walleye for a total of 5 hours, the solid line denotes water parameters in the middle transportation tank which transported Walleye for a total of 3 hours, and the line with triangles denotes water quality parameters in the front transportation tank which transported Walleye for 0.05 hours.

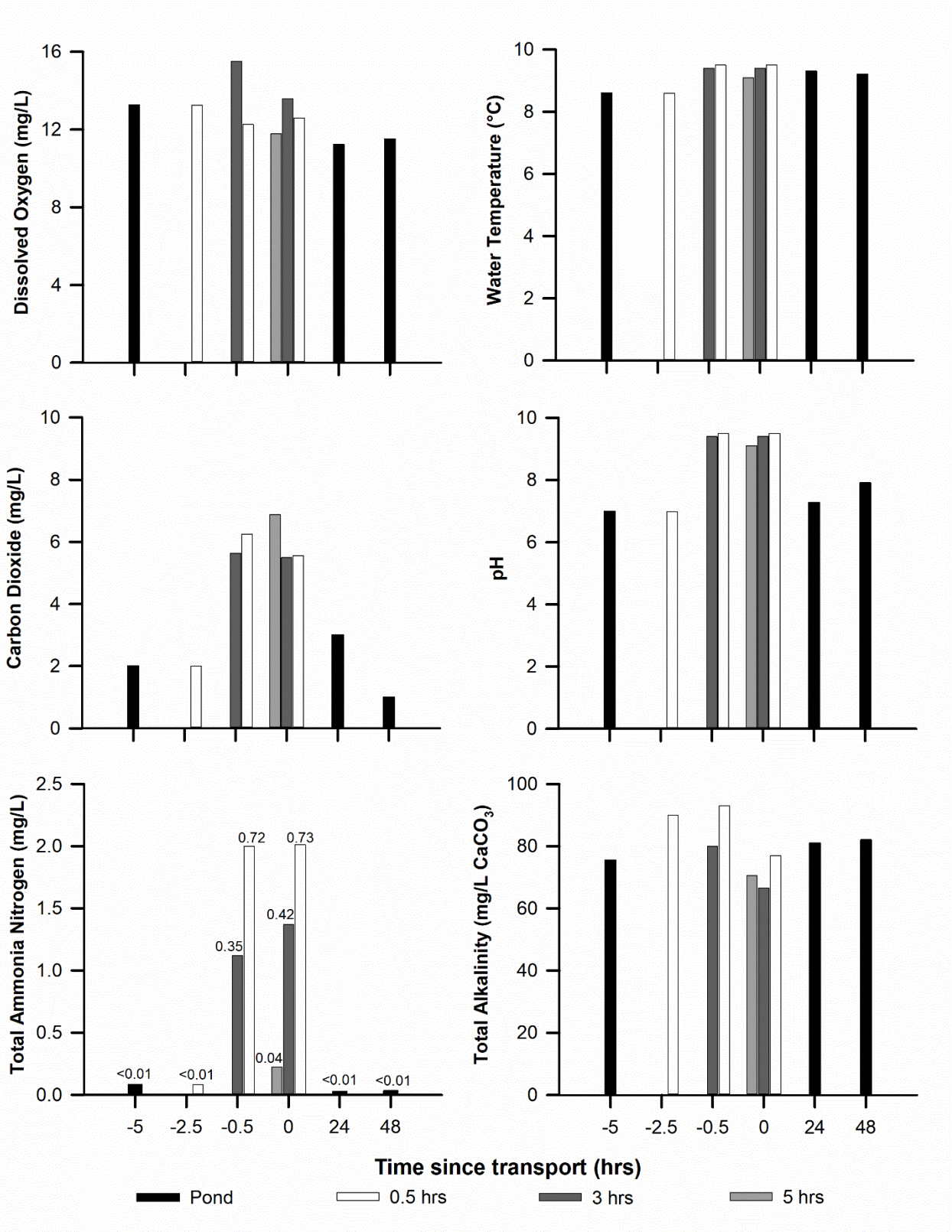
Dissolved Oxygen (mg/L)
Water Temperature (°C)
Carbon Dioxide (mg/L)
pH
Total Ammonia Nitrogen (mg/L)
Total Alkalinity (mg/L CaCO3)
<0.01
<0.01
0.35
0.72
0.04
0.42
0.73
<0.01
<0.01
-5
-2.5
-0.5
0
24
48
Time since transport (hrs)
Pond
0.5 hrs
3 hrs
5 hrs

Figure 3.2 Changes in dissolved oxygen (mg/L), temperature (˚C), carbon dioxide (mg/L), pH, total ammonia nitrogen (mg/L), and total alkalinity (mg/L $CaCO_3$) during and two days after transportation. Calculated un-ionized ammonia concentrations (mg/L) are denoted above bars on the total ammonia nitrogen panel. Each bar represents a different water type (e.g., ponds 1 & 2 = black, white = rear transportation truck tank, light grey = front transportation truck tank, and dark grey = middle transportation truck tank). Time since transport values is relative to the time when the transportation truck completed the five-hour transport duration (time since transportation = 0 hours).

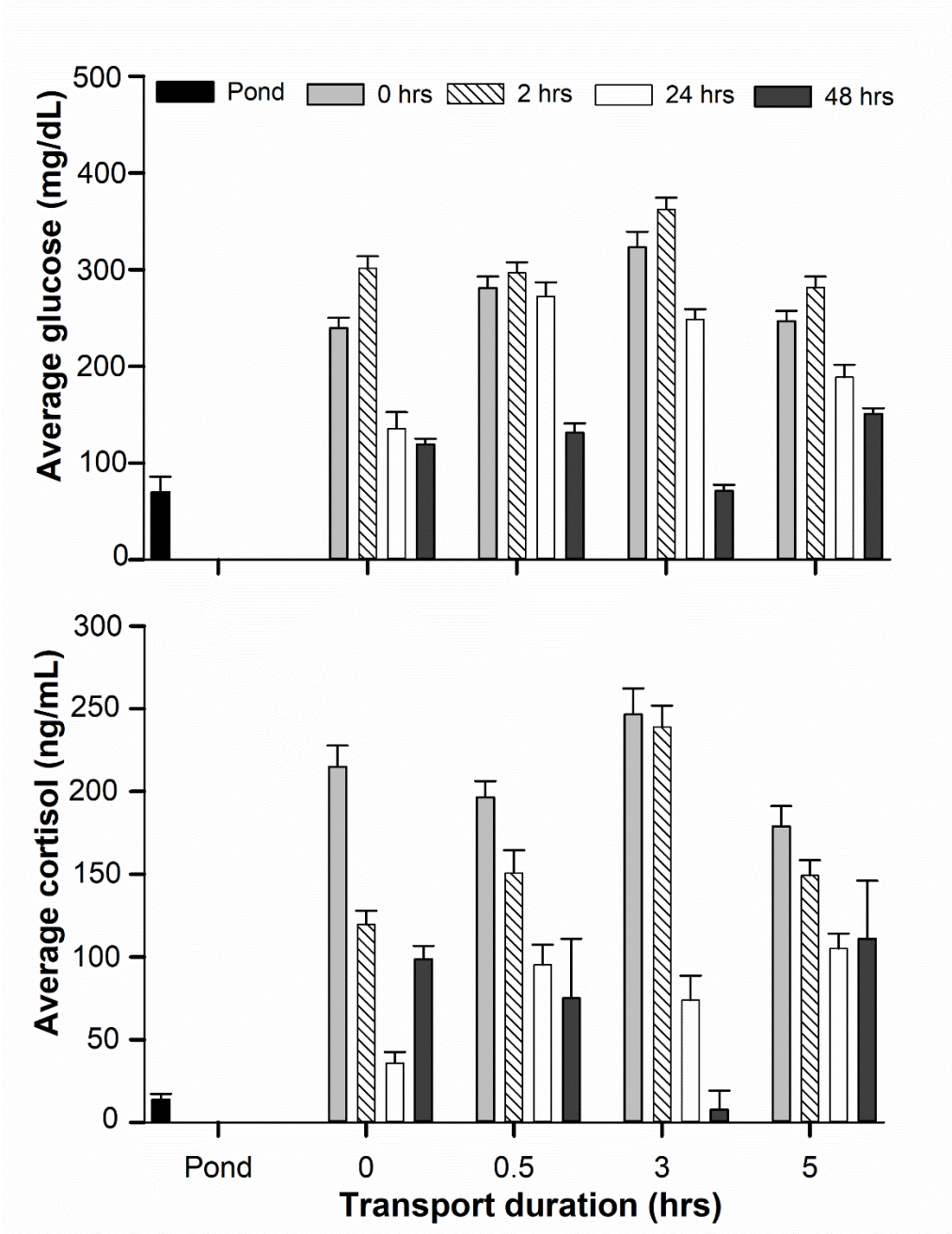

Figure 3.3 Comparison of average whole blood glucose (± SE) and plasma cortisol (± SE) concentrations of Walleye *Sander vitreus* exposed different transportation durations (0, 0.5, 3, and 5 hours) at different post-stocking monitoring times (reference Walleye = black; 0 hours = light grey; 2 hours = white with diagonal stripes; 24 hours = white; 48 hours = dark grey). The error bars represent standard error (SE) values of whole blood glucose and plasma cortisol.

CHAPTER 4. IS BIGGER BETTER? EVALUATION OF SIZE-SELECTIVE PREDATION ON AGE-0 WALLEYE *SANDER VITREUS*

Modified from a manuscript in press at North American Journal of Fisheries Management

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Abstract

The success of fish stocking programs is dependent on post-stocking survival. However, survival can be influenced by size-selective mortality, where larger individuals are expected to be less vulnerable to predation. Walleye *Sander vitreus* is a commonly stocked sportfish, but little is known regarding the role of size-selective predation on age-0 Walleye (90-290 mm) stocked in the presence of various piscivores with different foraging strategies and morphological features. Our objective was to evaluate whether consumed age-0 Walleye total length was related to predator total length, predator gape height, or the probability of predation. We also assessed if length distributions varied among stocked, recaptured, and consumed age-0 Walleye. Largemouth Bass *Micropterus salmoides*, Smallmouth Bass *M. dolomieu*, Northern Pike *Esox lucius*, adult Walleye, and Muskellunge *E. masquinongy* food habits were collected from East and West Okoboji, Iowa, USA during fall 2015-2017. Over the study, 301 age-0 Walleye were recovered from 3,514 predator stomachs. Mean, maximum (85th percentile), and minimum (15th percentile) consumed age-0 Walleye total length were not related to predator total length or gape height ($P > 0.05$), but the probability of predation decreased by 0.02 for every 10 mm increase in age-0 Walleye total length. Length distributions indicated consumed Walleye were generally smaller, whereas recaptured Walleye tended to be larger than stocked fish. Our approach to evaluating post-stocking size-selective predation on age-0 Walleye furthers the

current understanding of the importance of size at stocking relative to age-0 Walleye stocking success and suggests that stocking Walleye >220 mm is economically advantageous for reducing predation risk.

Introduction

Stocking is an important management tool for maintaining recreational fisheries and for sustaining declining populations of native species (Barton 2011). However, mortality rates of stocked fishes can vary widely (27-95%; Stein et al. 1981; Buckmeier et al. 2005; Freedman et al. 2012; Weber et al. 2020) and small changes in survival rates can result in large differences in year-class strength and success of stocking initiatives. Predation by piscivorous fishes can be a significant source of mortality for stocked fishes (Stein et al. 1981; Murphy and Kelso 1986). Predator-prey interactions are species-specific in part due to variable predator morphological features (e.g., total length and gape height; Scharf et al. 2000; Dörner and Wagner 2003) and foraging behaviors (e.g., sit-and-wait versus active foraging; Cooper 1995) as well as variability in prey morphology (e.g., body shape and behavior; Nilsson et al. 1995; Nilsson and Brönmark 2000). Therefore, species-specific evaluations regarding predator-prey relationships are crucial to enhancing stocking practices and improving fisheries.

The growth-predation hypothesis predicts that selective mortality should decline as individuals grow and increase in size (Anderson 1988). Increases in size are also associated with decreased predation risk (Post and Evans 1989; Miranda and Hubbard 1994), where larger body size can reduce the chances of predation due to improved maneuverability, swimming speed (Videler 1993), and predator gape limitations (Hyvärinen and Vehanen 2004). Thus, larger individuals should have higher survival relative to smaller individuals, which has been observed in Japanese Anchovy *Engraulis japonicus*, Walleye *Sanders vitreus*, Largemouth Bass *Micropterus salmoides*, and Northern Pike *Esox lucius* (Takasuka et al. 2003; Gaeta et al. 2018;

Weber et al. 2020), among many other fishes. Predation vulnerability can also be influenced by predator morphological features (e.g., total length and gape height; Scharf et al. 2000; Dörner and Wagner 2003) and foraging behavior (e.g., sit- and- wait versus active foraging; Cooper 1995). Understanding mechanisms underlying size-selective predation of stocked fishes by a suite of piscivores will aid in making informed stocking decisions based on the importance of stocked fish size relative to predation risk as well as costs associated with rearing progressively larger hatchery fish.

Walleye is a popular sport fish throughout North America and many populations are maintained through stocking (Barton 2011). However, Walleye stocking success can be erratic, in part due to post-stocking predation (Fielder 1992; Jennings and Philipp 1992; Mitzner 1992). Largemouth Bass (Santucci and Wahl 1993; Freedman et al. 2012; Kelling et al. 2016), Smallmouth Bass *Micropterus dolomieu* (Johnson and Hale 1977; Liao et al. 2004), Northern Pike (Anthony and Jorgensen 1977; Nate et al. 2003), and Muskellunge *Esox masquinongy* (Bozek et al. 1999) have been observed to consume age-0 Walleye and may negatively influence Walleye recruitment (Fayram et al. 2005). However, it is unclear whether predation on age-0 Walleye is dependent on age-0 Walleye total length relative to predator total length and/or predator gape height. Furthermore, it is unclear whether these relationships vary among predator species.

Our research objective was to evaluate size-selective predation by Largemouth Bass, Smallmouth Bass, Northern Pike, Muskellunge, and adult Walleye on fall stocked age-0 Walleye. Specifically, we evaluated whether consumed age-0 Walleye total length was related to predator total length, predator gape height, and the probability of predation. Second, we compared length-frequency distributions of stocked age-0 Walleye to distributions of age-0

Walleye recaptured during fall sampling and age-0 Walleye recovered from the stomachs of predators to assess whether predators were consuming sizes of Walleye that made up larger proportions of the stocked population. We hypothesized that total length of age-0 Walleye in predator diets would be positively related to predator total length and gape height, but that age-0 Walleye total length would be negatively related to the probability of predation. We also hypothesized that total length distributions of stocked and recaptured age-0 Walleye would be wider than consumed age-0 Walleye. Collectively, our results will provide insights as to whether size-specific post-stocking predation has the potential to negatively influence age-0 Walleye stocking success.

Methods

Age-0 Walleye stocking

Approximately 30,000 age-0 Walleye were annually stocked in East Okoboji and West Okoboji lakes in northwestern Iowa, USA, during fall 2015, 2016, and 2017 (2015: 31,440; 2016: 28,410; 2017: 30,461). Total length (mm) was measured on a subset of the stocked population each year one week prior to stocking (4,000 individuals; 13-14% of the stocked population). Age-0 Walleye total length ranged from 90-290 mm and the length distribution was bimodal, with distribution peaks at 180 mm and 220 mm (Figure 4.1). Age-0 Walleye were not fed 48 h prior to transportation in order to decrease waste (e.g., ammonia and carbon dioxide) during transportation (Robb 2008). The transportation truck had three 1,260-L compartments equipped with ram-air ventilation and supplemental oxygen (0.2 to 0.4 L min-1 with a maintained tank pressure at 276 kPa). Approximately 514 age-0 Walleye (60.7 kg ± 0.25 kg) were transported in each tank during each stocking event. Age-0 Walleye were stocked mid-afternoon from a single boat ramp via wet transfer each year.

Sampling processing

Predators (Largemouth Bass, Smallmouth Bass, Northern Pike, Muskellunge, and adult Walleye) and age-0 Walleye were sampled via nighttime boat electrofishing (220V, 5-8 amps pulsed DC) and experimental monofilament gillnetting (height: 1.5 m; length: 30 m; panel length: 3 m; soak time: 1-2 h; panel mesh: 37, 58, 25, 105, 45, 19, 65, 33, 76, 50 mm) in each system 14 d prior to and after the initial stocking event until ice-up (2015: 55 days; 2016: 54 days; 2017: 45 days). Age-0 Walleye collected alive via boat electrofishing after stocking are referred to as recaptured, whereas those collected from predator stomachs are referred to as consumed individuals hereafter. Stomach samples were collected across approximately 23 nights each year (2015: 22 nights; 2016: 23 nights; 2017: 24 nights). We used both boat electrofishing and gillnets during fall 2015 (September 22nd to November 20th), but only boat electrofishing was conducted during fall 2016 and 2017 (2016: September 13th to November 16th; 2017: September 14th to November 14th) due to low gillnet catch rates during 2015. The weekly average (95% CI) boat electrofishing shock time was 9.6 (± 3.7) hr. Upon capture, predator total length (mm) and gape height (mm; maximum linear distance between the upper and lower jaws with the mouth stretched open with a caliper) were measured, and stomach contents were non-lethally collected from all captured predators via pulsed gastric lavage (Foster 1977; Waters et al. 2004). Regurgitated contents from predators were concentrated on 150-µm mesh screen; non-age-0 Walleye prey items were preserved with 95% ethanol, and consumed age-0 Walleye were frozen (Brosse et al. 2002). Morphometric measurements (e.g., spinal column, anterior end of rostrum to margin of dorsal fin, and anterior end of rostrum to posterior margin of operculum) were collected from consumed age-0 Walleye to directly measure or back-calculate total length at the time of consumption using previously developed regression equations (Ball and Weber 2018).

Statistical analysis

We used linear regression and quantile regressions to assess the relationships between age-0 Walleye total length with either predator total length and gape height (Dörner and Wagener 2003; Krebs and Turingan 2003). Prey-predator total lengths can be polygonal in shape with upper and lower size boundaries changing at different rates (Scharf et al. 1998); thus, we analyzed the data by evaluating changes in the 85^{th} quantile and 15^{th} quantile using quantile linear regression (Scharf et al. 2000; Cade and Noon 2003). 85^{th} and 15^{th} quantiles were based on the adult Walleye sample size (n = 39); thus, each quantile regression line would be dependent on at least five data points (Scharf et al. 2000). Slope estimates of the 85^{th} and 15^{th} quantiles and the linear regression were compared using package "quantreg" in R 3.2.2 (Koenker 2018; R Core Team 2019) using a variant of the Wald test. The null hypothesis of this test was that the slope coefficient of the models were identical. We did not compare slope estimates for Smallmouth Bass (N=7) and Muskellunge (N=3) data due to small sample sizes. Logistic regression of total lengths of consumed (166 individuals) and recaptured (1,932 individuals collected alive in the field) age-0 Walleye were used to determine predation probability as a function of age-0 Walleye total length (Hosmer and Lemeshow 2013).

We used a two-sample Kolmogorov-Smirnov test (K-S Test) to compare total length distributions of stocked age-0 Walleye, age-0 Walleye recovered from predator diets, and age-0 Walleye recaptured alive after stocking. *P*-values for the K-S Test were computed using an asymptotic distribution when length-frequency bins had ties. Three separate K-S Tests were ran in order to make all possible comparisons across the three aforementioned distributions. Our null hypothesis was that length-frequency distributions of age-0 Walleye recovered from predator stomachs, recaptured alive, and those stocked all have similar distributions. K-S tests were conducted using Program R 3.2.2 at a significance level of $\alpha = 0.05$ for all analyses.

Results

Over the three-year study period, we recaptured 1,932 age-0 Walleye during boat electrofishing as well as captured 1,906 Largemouth Bass, 85 Muskellunge, 820 Northern Pike, 66 Smallmouth Bass, and 637 adult Walleye. No age-0 Walleye were recovered from predator diets or recaptured from West Okoboji with boat electrofishing prior to fall stocking. However, 64 age-0 Walleye were collected in 2016, and six were collected in 2017 via boat electrofishing in East Okoboji prior to stocking. A total of 301 age-0 Walleye were recovered from predator stomachs during fall 2015-2017, with the number of age-0 Walleye recovered varying across species (Largemouth Bass = 124; Muskellunge = 4; Northern Pike = 98; Smallmouth Bass = 11; and adult Walleye = 64). We were able to directly measure total length at time of consumption for 126 age-0 Walleye (adult Walleye = 39; Largemouth Bass = 47; Muskellunge = 3; Northern Pike = 70; Smallmouth Bass = 7; Figure 4.2) whereas back-calculation techniques were used to determine total lengths for an additional 40 age-0 Walleye recovered from predator stomachs (24.1%; 166 Walleye with length measurements in total).

Consumed age-0 Walleye were recovered from a range of predators with variable total lengths (adult Walleye: 430-680 mm; Largemouth Bass: 300-557 mm; Muskellunge: 872-930 mm; Northern Pike: 412-888 mm; Smallmouth Bass: 354-421 mm; Figure 4.2A), and predators consumed a range of age-0 Walleye lengths (Largemouth Bass: 120-253 mm; Muskellunge: 140-185 mm; Northern Pike: 78-295 mm; Smallmouth Bass: 95-208 mm; Walleye: 94-242 mm; Figure 4.2B). Northern Pike consumed age-0 Walleye that were 20-70% of their total lengths, Largemouth and Smallmouth Bass consumed age-0 Walleye 10-60% of their total length, and adult Walleye consumed age-0 Walleye 20-40% of their total length. However, predator total length (mm; Figure 4.3) was not related to the total length of consumed age-0 Walleye (Largemouth Bass: $R^2 < 0.001$, N = 47, $P = 0.92$; Northern Pike: $R^2 = 0.03$, N = 70, $P = 0.18$;

Smallmouth Bass $R^2 = 0.09$, N = 7, $P = 0.52$; adult Walleye: $R^2 < 0.001$, N = 39, $P = 0.93$). Furthermore, slopes of the 85th, 50th, and 15th linear regressions between predator and age-0 Walleye total length were not significantly different for adult Walleye ($F_{2,155} = 0.999$; $P = 0.37$), Northern Pike ($F_{2,211} = 0.253$; $P = 0.78$), and Largemouth Bass ($F_{2,142} = 0.142$; $P = 0.87$). Similarly, predator gape height (mm) was not related to the total length of consumed age-0 Walleye (Largemouth Bass: $R^2 = 0.30$, N = 47, $P = 0.23$; Northern Pike: $R^2 = 0.02$, N = 70, $P = 0.22$; Smallmouth Bass $R^2 = 0.29$, N = 7, $P = 0.21$; adult Walleye: $R^2 < 0.001$, N = 39, $P = 0.93$). Slopes of the 85th, 50th, and 15th linear regressions between predator gape height and age-0 Walleye total length were not significantly different for adult Walleye ($F_{2,12} = 0.500$; $P = 0.61$), Northern Pike ($F_{2,202} = 1.267$; $P = 0.28$), and Largemouth Bass ($F_{2,142} = 0.375$; $P = 0.69$; Figure 4.3). Across all predators, the odds of predation decreased by 0.02 for every 10 mm increase in age-0 Walleye total length (Figure 4.4; $Z = -7.62$; $P < 0.0001$). Age-0 Walleye 150 mm had an 18.8% chance of being consumed while those 200 mm had less than a 7.1% chance of being consumed (Figure 4.4).

Distributions of consumed, recaptured, and stocked age-0 Walleye all included age-0 Walleye with total lengths less than 150 mm. Specifically, stocked age-0 Walleye had individuals with total lengths of 90-290 mm and recaptured age-0 Walleye had individuals with total lengths 110-300 mm; however, smaller individuals (<150 mm) in the stocked and recaptured distributions made up ≤ 1% of their respective group. For consumed age-0 Walleye, individuals 90-140 mm made up a large percentage of that group of age-0 Walleye (11%). Generally, larger (>220 mm) age-0 Walleye were more frequently recaptured than consumed (Figure 4.1). However, KS tests indicated that length distributions of stocked age-0 Walleye were not statistically different from consumed (D = 0.29, $P = 0.26$) and recaptured (D = 0.17, P

= 0.89) age-0 Walleye (Figure 4.1). Additionally, length-frequency distributions of recaptured and consumed age-0 Walleye were also statistically similar (D = 0.21, *P* = 0.67; Figure 4.1).

Discussion

The relationship between prey and predator size has long been recognized as being important in determining the outcome of interactions among species. However, our hypothesis regarding positive relationships between age-0 Walleye total length and predator total length or gape height was not supported with the minimum (15^{th} quantile), mean, or maximum (85^{th} quantile) linear regressions. While larger adult Walleye, Largemouth Bass, and Northern Pike have been reported to have diets consisting of higher proportions of larger prey (Gaeta et al. 2018), lack of relationship between predator morphometric features and age-0 Walleye total length could be related to foraging success, as predator capture success decreases monotonically with increasing prey size for a wide variety of fishes (Ellis and Gibson 1997; Scharf et al. 1998). Thus, predators may select for smaller prey items in order to increase capture success rates (Juanes and Conover 1994; Weber et al. 2011) as well as avoid predation and/or competition (Nilson and Brönmark 1999). Specifically, Northern Pike have been observed to preferentially consume prey far smaller than ingestible, and even smaller than predicted from optimal foraging theory, potentially due to risks associated with competition and capture success (Juanes and Conover 1994; Nilsson and Brönmark 1999, 2000). Competitive interactions among predators have been observed where larger individuals initially consume smaller prey items to outcompete smaller conspecifics that are limited by appropriately sized prey (e.g., size-efficiency hypothesis; Brooks and Dodson 1965; Scharf et al. 2000). While we did not show a direct relationship between age-0 Walleye total length and predators' morphometric measurements (e.g., gape height and total length), we did observe that predation risk decreased with increasing age-0 Walleye total length. Thus, from a hatchery production and stocking perspective, stocking larger

individuals may decrease predation mortality on stocked age-0 Walleye and increase stocking success.

Despite KS analyses indicating that length distributions of stocked, recaptured, and consumed Walleye were statistically similar, visual assessment of length-frequency distributions suggests that predators more frequently consumed smaller individuals and less often consumed Walleye >200 mm. KS tests are sensitive to the center of the distribution and have difficulties in detecting differences in distribution tails (Stephens 1992), likely limiting their ability to detect differences in age-0 Walleye length distributions here. Potential size-selective predation was further supported by our logistic regression analysis that indicated predation probability decreased with age-0 Walleye total length. Predation on the smallest individuals within a cohort has also been observed for flatfish species *Pleuronectes sp.*, Northern Pike, Muskellunge, and Tiger Muskellunge *Esox masquinongy x Esox lucius* (Wahl and Stein 1989; Ellis and Gibson 1995). Therefore, despite a lack of statistical differences, length-frequency distributions of stocked, recaptured, and consumed Walleye along with our logistic regression analysis suggest that piscivores have diets consisting more frequently of smaller age-0 Walleye.

Stocking is an important management tool for maintaining recreational fisheries and for replenishing Walleye populations. However, hatchery production is an expensive process that depends on a variety of factors, such as the size of the operation, personnel costs, feed, medication, heating costs, etc. Additionally, production costs are generally positively related to the size of fish (Margenau 1992; Fenton et al. 1996). For example, the average cost of an age-0 Walleye from 2015-2018 produced at Spirit Lake Fish Hatchery at an average size of 183 mm was $0.93 USD per fish whereas the average cost at Rathbun Fish Hatchery at an average size of 234 mm was $1.27 per fish (P. Surber, Iowa DNR, personal communication), a difference of

$0.34. While direct cost analyses of fry, fingerling, and advanced fingerling stockings have been conducted and are relatively straightforward, cost-analyses of age-0 Walleye that are cultured for the same amount of time but reach different sizes (e.g., 200 versus 220 mm) due to differences in individual foraging rates, growth rates, etc. is much more complicated. Nonetheless, the production cost of different sizes of age-0 Walleye at these two hatcheries suggests that producing larger age-0 Walleye in some instances may be a worthy investment due to decreased predation probability as well as potential increases in survival, recruitment, and return to the fishery. However, a detailed analysis to determine if the benefits of producing larger age-0 Walleye outweigh production costs was beyond the scope of the current study. For fisheries managers, the decision to produce and stock larger fish creates a balancing act between management needs and fish production capacity considering space to hold and production costs.

Post-stocking predation can negatively influence the success of age-0 Walleye stocking initiatives. While morphological features (total length and gape height) of predators were not directly related to consumed age-0 Walleye total length, our results indicate that smaller age-0 Walleye (< 220 mm) had a higher probability of being consumed by predators. Similarly, Largemouth Bass were estimated to consume 0% of large age-0 Walleye (185-216 mm), but 10-11% of small (48-61 mm) and 7-28% of medium (140-145 mm) age-0 Walleye stocked into a centrarchid dominated impoundment (Santucci and Wahl 1993). Collectively, our research, as well as prior research, indicates that larger age-0 Walleye generally have a lower risk of predation and maybe a more economically viable solution rather than stocking more smaller size classes of age-0 Walleye. However, unlike prior research, our results suggest that post-stocking predation age-0 Walleye is not influenced by predator morphometric features, such as total length or gape height. Therefore, stocking age-0 Walleye ≥ 220 mm TL has the potential to

improve age-0 Walleye stocking success in systems that historically had erratic post-stocking survival, partially due to post-stocking predation by a suite of predators.

Acknowledgments

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Figures

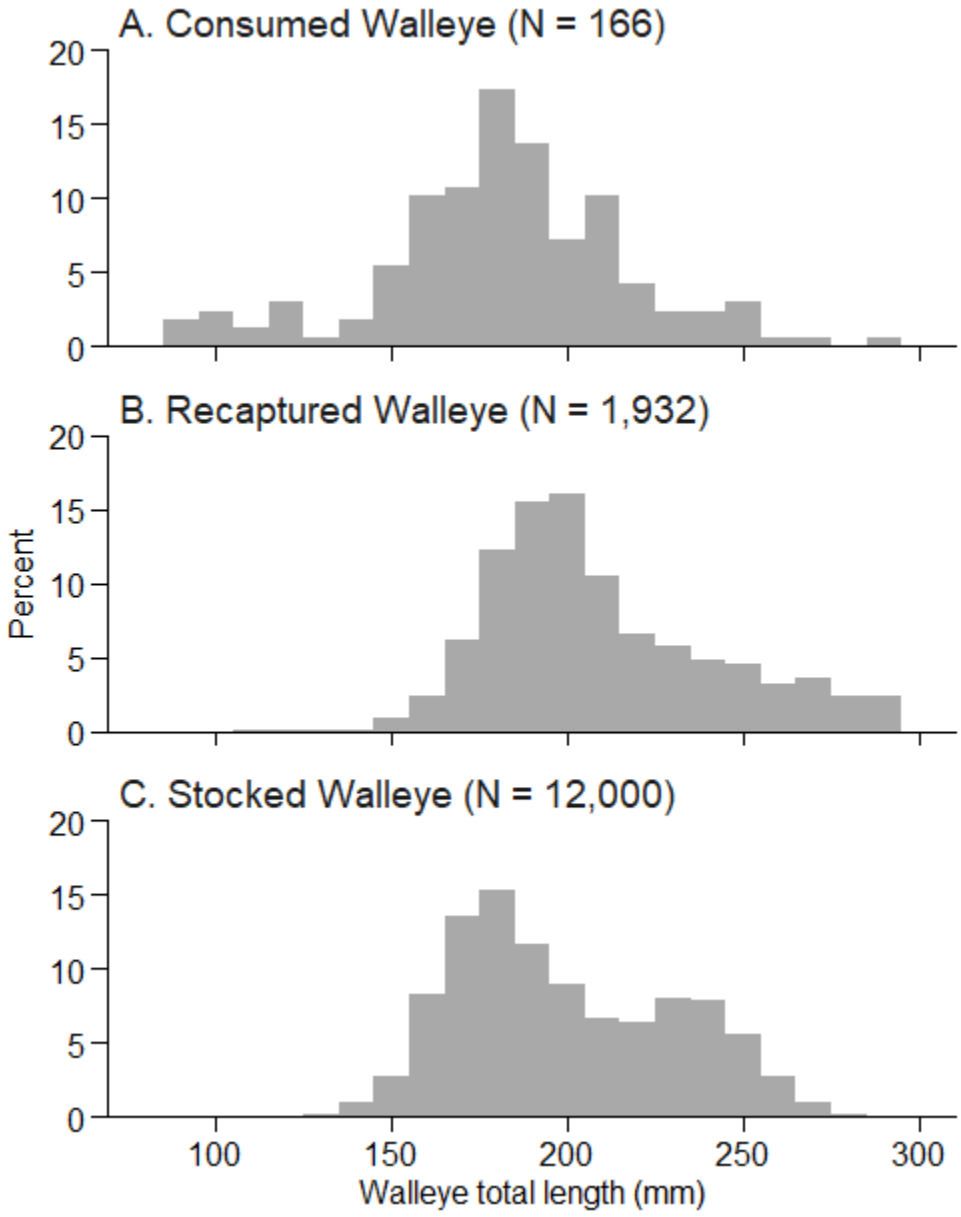

Figure 4.1 Length-frequency distributions of consumed (A), recovered (B), and stocked (C) age-Walleye across all years and predators sampled.

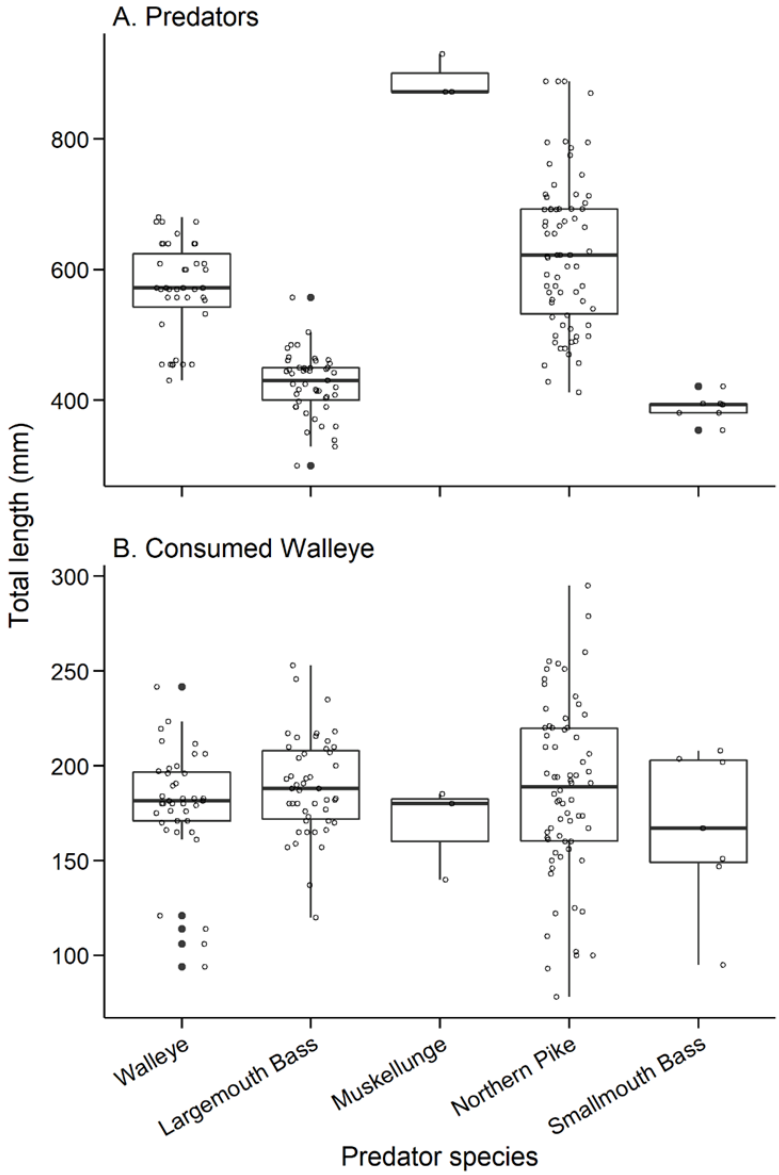

Figure 4.2 Boxplots of total lengths (mm) of adult Walleye, Largemouth Bass, Northern Pike, Muskellunge, and Smallmouth Bass (hollow circles) (A) and age-0 Walleye that they consumed (B). The whiskers (vertical black lines) of the boxplot extend up from the top of the box to the largest data point and down from the box the smallest data point which are not outlying values. Outlier values are represented by solid black dots. The black horizontal lines represent different percentiles (bottom = 25th; middle = 50th; top = 75th).

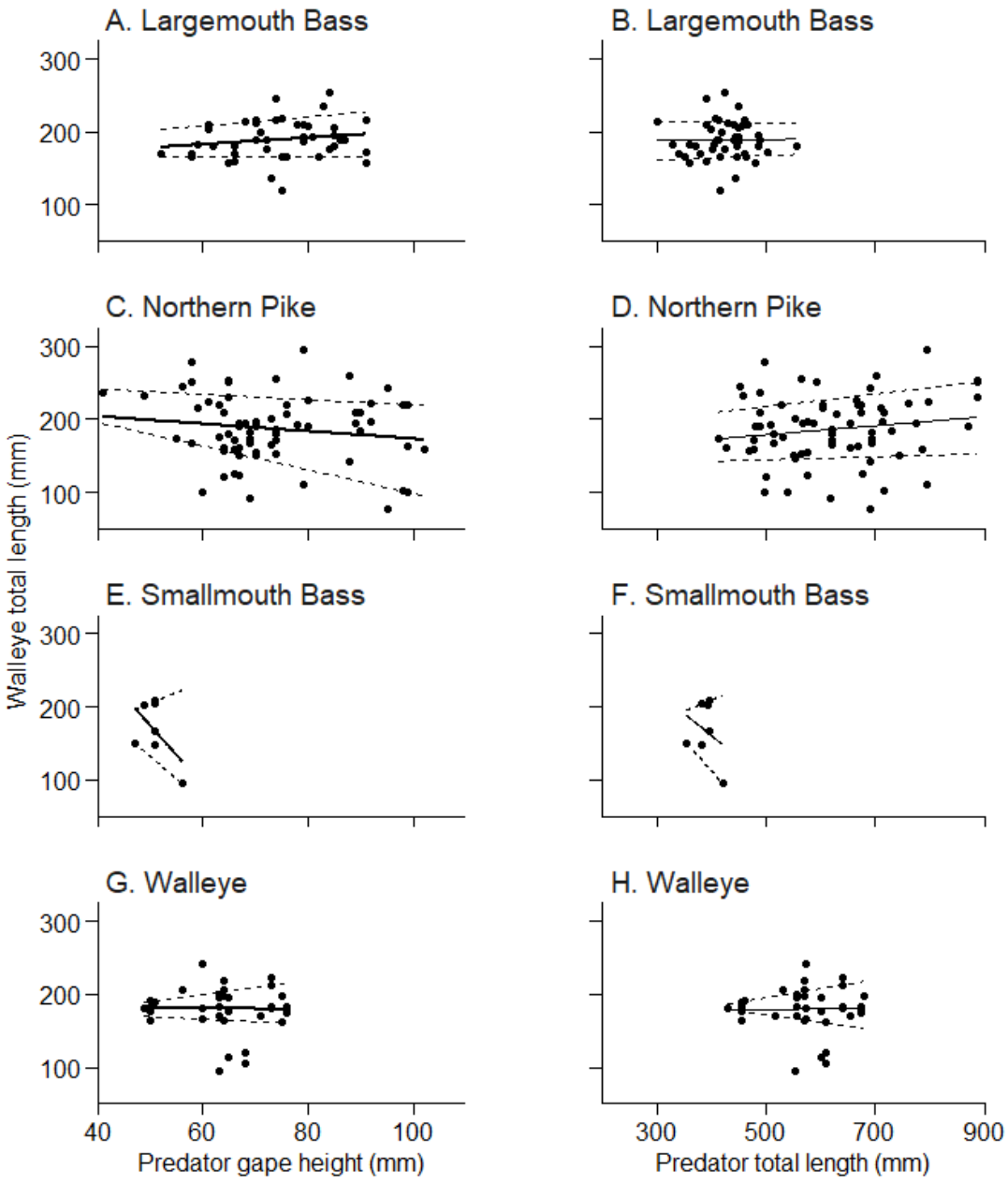

Figure 4.3 Linear regression (solid black line), 85th and 15th quantile regressions (black dotted lines) between consumed age-0 Walleye total length (mm; right) and total length (mm; right column) and gape height (mm; left column) of Largemouth Bass (A & B), Northern Pike (C & D), Smallmouth Bass (E & F), and adult Walleye (G & H). Linear regression was not included for Muskellunge (N=3) due to small sample size.

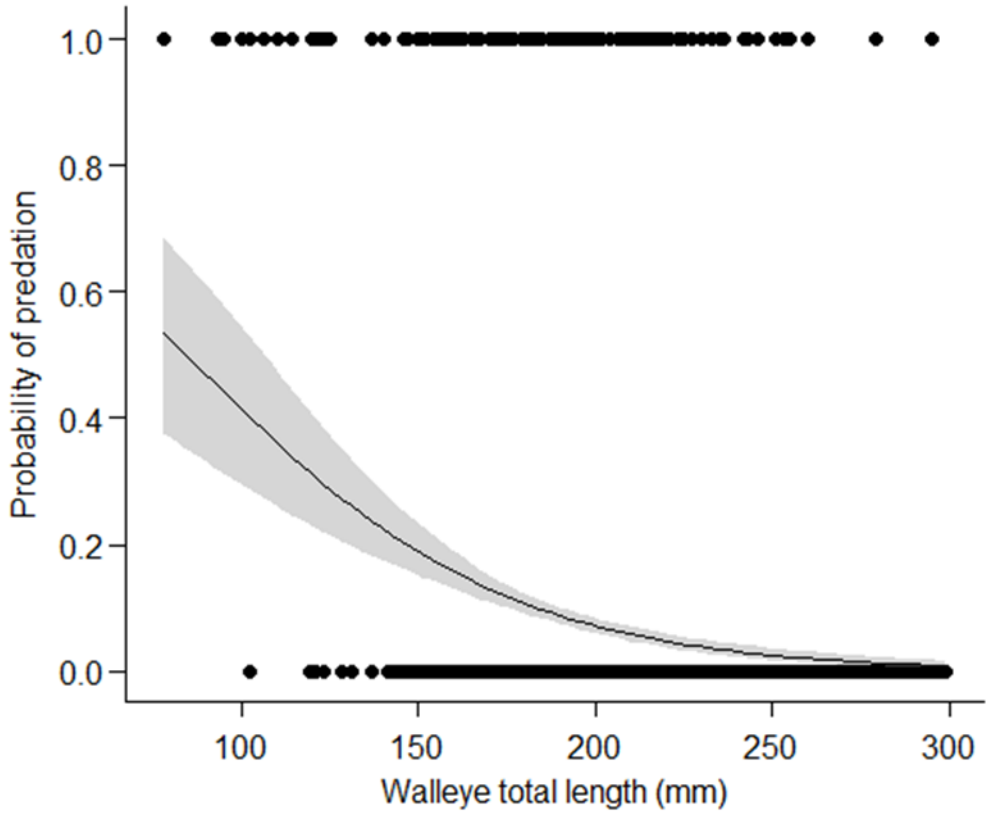

Figure 4.4 Logistic regression estimating the probability of predation versus age-0 Walleye total length (mm). Age-0 Walleye with a value of 1 were consumed while age-0 Walleye with a value of 0 were not consumed. The solid black line represents the logistic regression curve and the shaded grey area indicates the 95% confidence intervals for the regression line.

CHAPTER 5. USING BIOENERGETICS TO ESTIMATE CONSUMPTION OF STOCKED AGE-0 WALLEYE *SANDER VITREUS* BY A SUITE OF PISCIVORES

Modified from a manuscript under review to North American Journal of Fisheries Management

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Abstract

Age-0 Walleye *Sander vitreus* have been stocked at progressively larger sizes to improve survival and stocking success; however, the effects of post-stocking predation are uncertain for larger (>200 mm) individuals. Our objective was to evaluate post-stocking predation on age-0 Walleye (78-295 mm) and estimate cumulative consumption for up to two months by a suite of piscivores. Largemouth Bass *Micropterus salmoides*, Northern Pike *Esox lucius*, and adult Walleye diets were collected from East Okoboji and West Okoboji lakes, Iowa, USA. Schnabel models were used to estimate predator population size and bioenergetics models were used to determine the biomass of consumed age-0 Walleye from stocking through ice-up. Across years and systems, we collected 3,272 predators during fall 2017 and 2018, from which 257 age-0 Walleye were recovered. Northern Pike (0.12 Walleye per predator ± 0.32 SD) had the highest proportion of age-0 Walleye in their diets followed by Largemouth Bass (0.11 ± 0.30 Walleye per predator) and adult Walleye (0.04 ± 0.20 Walleye per predator). Largemouth Bass, Northern Pike, and adult Walleye were collectively estimated to consume between 2.4-27.2% of the stocked age-0 Walleye within one month of stocking, with higher proportions of smaller (<220 mm) Walleye in predator diets. In East Okoboji, the highest proportions of age-0 Walleye in predator diets generally occurred 14 days after the most recent stocking event with the proportion of age-0 Walleye in predator diets decreasing thereafter. However, in West Okoboji, Northern

Pike, adult Walleye, and Largemouth Bass had increasing proportions of age-0 Walleye in diets between stocking events. Collectively, local piscivores have the potential to consume more than a quarter of stocked age-0 Walleye within the first two months of stocking, suggesting that managers could consider alternative stocking practices to decrease post-stocking predation.

Introduction

Stocking is an important management tool for maintaining recreational fisheries and for replenishing declining populations of native species (Kerr 2011). However, mortality rates of stocked fishes can be highly variable (e.g., 27-95%; Stein et al. 1981; Buckmeier et al. 2005). Thus, small changes in post-stocking survival rates can significantly influence the success of stocking initiatives. Post-stocking predation can be a significant mortality source for stocked fishes (Stein et al. 1981; Howell 1994; Hyvärinen and Vehannen 2004; Kekäläinen et al. 2008). However, post-stocking prey-predator interactions are species-specific and the length of time in which post-stocking predation is a concern can range from a few hours (Santucci and Wahl 1993; Henderseon and Letcher 2003; Buckmeier et al. 2005; Freedman et al. 2012) to 40 days (Stein et al. 1981; Baldwin et al. 2003). Thus, post-stocking evaluations assessing temporal trends in predation provide valuable insights as to whether post-stocking predation limits the success of stocking initiatives.

The growth-predation hypothesis predicts that size-selective mortality should decline as individuals grow and increase in size (Anderson 1988) due to improved maneuverability and swimming speed (Videler 1993). Additionally, as fish grow, they can outgrow the gape limitations of their predators (Hyvärinen and Vehanen 2004; Lawson and Carpenter 2014). The argument of “bigger-is-better” (Butler 1988; Miller et al. 1988; Litvak and Leggett 1992) has led hatcheries to raise progressively larger individuals (Halverson 2008). However, hatchery

production is an expensive and labor-intensive process, where production costs are generally positively related to rearing duration and fish size (Wedemeyer 2001). Therefore, it is advantageous to evaluate size-specific prey-predator relationships to assess whether rearing larger hatchery fish is justifiable.

Walleye *Sander vitreus* is a commonly stocked sportfish in the United States (Ellison and Franzin 1992; Fenton et al. 1996; Barton 2011) in part due to declining population abundance and variable natural reproduction (Kerr 2011; Hansen et al. 2015). Walleye are stocked at a variety of sizes (e.g., fry and fingerling) with variable stocking success (Fielder 1992; Jennings and Philipp 1992; Mitzner 1992; Kampa and Hatzenbelor 2009). Several predators have been documented to consume a wide size range of age-0 Walleye (16-300 mm), including Largemouth Bass *Micropterus salmoides* (Freedman et al. 2012; Grausgruber and Weber *in press*), Smallmouth Bass *Micropterus dolomieu* (Johnson and Hale 1977; Liao et al. 2004), Northern Pike *Esox lucius* (Anthony and Jorgensen 1977; Nate et al. 2003; Grausgruber and Weber *in press*), Muskellunge *Esox masquinongy* (Bozek et al. 1999; Grausgruber and Weber *in press*), and adult Walleye (Chevalier 1973; Liao et al. 2002; Grausgruber and Weber *in press*). However, prior approaches used to evaluate the effects of post-stocking predation are limited by their classification of age-0 Walleye into broad size classes (e.g., larval, small, medium, and large fingerlings; Santucci and Wahl 1993; Brooks et al. 2002; Hoxmeier et al. 2006), limited temporal resolution (Stein et al. 1981; Santucci and Wahl 1993; Freedman et al. 2012), and focus on Walleye stocked at smaller sizes (< 200 mm; Chevalier 1973; Hoxmeier et al. 2006; Freedman et al. 2012). Evaluating post-stocking predation on a fine temporal scale (e.g., daily predation trends) across a broader and more extensive size range of age-0 Walleye has the

potential to provide insights regarding whether age-0 Walleye raised in hatcheries to large sizes are still vulnerable to predation.

Our research objective was to estimate post-stocking predation on age-0 Walleye (78-295 mm; 12-253 g) by a suit of predators (e.g., Largemouth Bass, Northern Pike, and adult Walleye). Specifically, we were interested in determining what percentage of stocked age-0 Walleye were consumed by predators, size-specific consumption of stocked age-0 Walleye, and temporal trends in consumption rates across predators. We hypothesized that the proportion of age-0 Walleye in diets would vary across predator species, systems, and years. We also hypothesized that predators would have higher proportions of smaller age-0 Walleye relative to larger age-0 Walleye. Finally, we hypothesized that consumption of age-0 Walleye would initially be elevated and then decrease after a stocking event, with predators consuming roughly 15% of the stocked Walleye. Collectively, these results provide insights as to whether several piscivorous species (Largemouth Bass, Northern Pike, and adult Walleye) have the potential to negatively influence age-0 Walleye stocking success and provide informative data that will aid fisheries managers in making informed stocking decisions.

Methods

Study systems

East Okoboji and West Okoboji are located in Dickinson County, Iowa. East Okoboji is a long, narrow, and shallow eutrophic natural lake (743 ha) with a 4,767-ha watershed that consists primarily of agriculture. The lakes basin slopes gradually and has a mean depth of 3.2 m and a maximum depth of 6.7 m. West Okoboji is an extensive oligotrophic system (1,557 ha) with a 6,133-ha watershed that consists primarily of agriculture. Relative to East Okoboji, West Okoboji has a more complex basin with a mean depth of 11.6 m and a maximum depth of 40.8

m. East Okoboji and West Okoboji are interconnected via a canal located on the southeast side of West Okoboji. Submersed aquatic vegetation abundant in both East Okoboji and West Okoboji includes Wild Celery *Vallisneria americana*, Flatstem Pondweed *Potamogeton zosteriformis*, Curly-leaf Pondweed *Potamogeton crispus*, Bushy Pondweed *Najas flexillis*, Clasping Leaf Pondweed *Potamogeton richardsonii*, and Coontail *Ceratophyllum demersum*. Curly-leaf Pondweed dominates the upper two basins of East Okoboji from late fall to early summer. Piscivore assemblages in East Okoboji and West Okoboji include Largemouth Bass, Muskellunge, adult Walleye, Northern Pike, and Smallmouth Bass.

Age-0 Walleye stocking

Age-0 Walleye (78-295 mm; 12-253 g) were stocked in East Okoboji and West Okoboji in northwestern Iowa, USA, during fall 2016 and 2017. Each year, approximately 30,000 age-0 Walleye were stocked (2016: 28,410; 2017: 30,461) across both systems. Age-0 Walleye reared at the Spirit Lake Hatchery were stocked in late September while age-0 Walleye reared at the Rathbun Hatchery were stocked in late October (Table 5.1). One week before stocking, total length (mm) and wet weight (g) was determined for a subset of the stocked population (4,000 individuals per year; 13-14% of the stocked population). Wet weight and total length values from the subset of stocked individuals were used to develop an exponential relationship that could be used to determine the wet weight of consumed age-0 Walleye:

$$W = 4.117e^{0.0139TL}$$

where *TL* is the total length of an age-0 Walleye (mm) and *W* is the wet weight of a non-consumed age-0 Walleye (g). Age-0 Walleye were not fed 48 hours before transportation to decrease waste (e.g., ammonia and carbon dioxide) during transport (Robb 2008). The transportation truck had three 1,260 L compartments, all equipped with ram-air ventilation and

supplemental oxygen (0.2 to 0.4 L min-1 with a maintained tank pressure at 276 kPa). Approximately 514 age-0 Walleye (60.7 kg ± 0.25 kg) were transported in each tank during each stocking event and stocked mid-afternoon off the boat ramp via wet transfer.

Sample collection and processing

Largemouth Bass, Northern Pike, and adult Walleye were sampled via nighttime boat electrofishing (220 V, 5-8 amps pulsed DC) in each system during fall 2016 and 2017 (2016: September 13th to November 16th; 2017: September 14th to November 14th). This research was done under the guidance and approval from Iowa State University Institutional Animal Care and Use Committee (Iowa State University IACUC 7-15-8051-I). Sampling was done two weeks before stocking to evaluate pre-stocking predator diets (2016: 13 days; 2017: 14 days). Due to the large size of the systems (East Okoboji = 743 ha; West Okoboji = 1,557 ha) and limited post-stocking dispersal of age-0 Walleye (Weber et al. 2020), our sampling focused in a 229-ha area in East Okoboji and a 152-ha area in West Okoboji, with additional sampling lake-wide that was more limited in frequency. Boat electrofishing consisted of sampling the entire circumference of the 229-ha area in East Okoboji and a 152-ha area in West Okoboji with boat electrofishing taking place in depths ≤ 3.5 m. Boat electrofishing run duration was variable, as we did not want to hold fish in high densities for a prolonged duration since stressed fish will regurgitate stomach contents. Upon capture, predator total length (mm) and weight (g) were recorded, and stomach contents were non-lethally collected from all captured predators via pulsed gastric lavage (Foster 1977; Waters et al. 2004). Regurgitated contents from predators were concentrated on a 150-µm mesh screen, with non-Walleye prey items being preserved with 95% ethanol whereas consumed age-0 Walleye were frozen. Consumed age-0 Walleye total length was either directly measured or back-calculated from morphometric measurements (e.g., spinal column, anterior end of

rostrum to margin of dorsal fin, and anterior end of the rostrum to posterior margin of operculum) allowing us to estimate total length at time of consumption (Ball and Weber 2018). After morphometric measurements were collected from consumed age-0 Walleye, all age-0 Walleye were preserved in 95% ethanol to ensure that all prey items recovered from predator diets underwent the same preservation techniques, reducing bias due to different preservation techniques on wet weight. All prey items were identified to the lowest taxonomic level and weighed. Prey items were then classified into the following groups: Ictaleuridae, Centrarchidae, Decapoda, Cyprinidae, unidentifiable fish, Sciaenidae, adult Anura, Clupeidae, Insecta, larval Anura, unidentifiable, age-0 Walleye, Moronidae, and Percidae (Table 5.2). Wet weights of individual prey were used to determine the proportion of prey in the diets of individual predators and included in bioenergetics models (Appendix A).

Predator population estimate

Upon each initial capture predators received a triangular fin clip (2016 = right pelvic fin; 2017 = top caudal fin) to identify the number of individuals recaptured more than one time. Species-specific predator population abundance and 95% confidence intervals of predators in East Okoboji and West Okoboji were estimated for 2016 and 2017 using Schnabel models calculated by ($\widehat{N}$; Schnabel 1938)

$$\widehat{N} = \frac{\sum_{i=9}^{t} n_i M_i}{\sum_{i=9}^{t} m_i + 1}$$

where *t* is the number of sampling occasions (≤ 9 weeks post-stocking), n_i is the number of fish caught in the *i*th sample, m_i is the number of fish caught with marks in the *i*th sample, and M_i is the number of marked fish present in the population of the *i*th sample. The variance estimator for the 95% confidence interval was

$$\hat{V}(N) = \widehat{N^2}\left[\frac{\hat{N}}{\sum n_i M_i} + 2 \cdot \frac{\hat{N}^2}{(\sum n_i M_i)^2} + 6 \cdot \frac{\hat{N}^3}{(\sum n_i M_i)^3}\right]$$

(Hayes et al. 2007). Assumptions of the Schnabel model include a closed population, all animals equally likely to be sampled, capture and marks do not influence catchability, marks are not lost, and all marks are recorded and reported (Hayes et al. 2007). Capture-recapture of predators occurred over a maximum of nine weeks to obtain reliable population estimates while maintaining a reasonable assumption of a closed population. However, deaths, emigration, or immigration occurring during this period could have resulted in under or overestimation of predator abundances that could have biased the estimates of consumed age-0 Walleye.

Describing predator diets

To evaluate whether the number of consumed prey categories varied across predator species, years, and systems, we used the packages 'mvabund' (Wang et al. 2019) with the 'manyglm' function in R 3.2.2 (Integrated development for R. R-Studio, Inc., Boston, USA). The packages were used to fit generalized linear models to our high-dimensional data (Warton 2011). The 'anova.manyglm' function was used to summarize the statistical significance of a fitted 'manyglm' model (Warton 2011). We used 999 bootstrap iterations, a negative binomial family-wise error rate, and individual predators were used as replicates (Warton 2011). The predictor variables included in each model were predator species (Largemouth Bass, Northern Pike, and adult Walleye), year (2016 and 2017), and system (East Okoboji and West Okoboji). The response variable was a matrix of the counts of categorized prey items (Ictaleuridae, Centrarchidae, Decapoda, Cyprinidae, unidentifiable fish, Sciaenidae, adult Anura, Clupeidae, Insecta, larval Anura, unidentifiable, age-0 Walleye, Moronidae, and Percidae). Post-hoc tests using Tukey's multiple comparisons correction were used to evaluate whether predators within a system during a given year had differing counts of prey items recovered from their stomachs.

Bioenergetics modeling

Bioenergetics modeling is a tool commonly used to assess predatory effects on prey populations (Hartman and Hayward 2007; Deslauries et al. 2017) and was used to estimate daily biomass of stocked age-0 Walleye consumed by each predator (Largemouth Bass, Northern Pike, and adult Walleye) within a system (East Okoboji and West Okoboji) from time of stocking through ice up during 2016 and 2017 (R package Fish Bioenergetics 4.0; Zweifel et al. 2010; Deslauriers et al. 2017). Several input files were required for running Fish Bioenergetics 4.0: (1) prey energy densities (J/g wet weight; Table 5.2), (2) predator energy densities (J/g wet weight), (3) water temperatures (°C), (4) proportions of consumed prey items, and (5) predator average initial and final weights (g). Each model input has to be on a similar temporal scale; thus, "Day 1" in all models was the first age-0 Walleye stocking event in each system (East Okoboji and West Okoboji) for each year (2016 and 2017). Prey and predator energy densities (Table 5.2) were obtained from published studies (Kelso 1972; Miranda and Muncy 1989; Bryan et al. 1996; Yako et al. 2000; Eggleton and Schramm 2002; Liao et al. 2004). Daily average water temperatures for each system were collected hourly by three centrally located HOBO Pendant loggers that were vertically suspended in the water column. The first temperature logger was located 1.5 m below the surface, the middle logger was located midway between the top logger and the third logger located 1 m above the sediment. The average of these three loggers was used for temperature in the bioenergetics model. Proportions of consumed prey categories were determined by dividing the wet weight of each recovered prey category (Ictaleuridae, Centrarchidae, Decapoda, Cyprinidae, unidentifiable fish, Sciaenidae, adult Anura, Clupeidae, Insecta, larval Anura [tadpoles], unidentifiable, age-0 Walleye, Moronidae, and Percidae) by the total wet weight of stomach contents recovered from individual predators. Average daily

proportions of consumed prey categories were incorporated into each bioenergetics model (Appendix A). Because the modeling was done on a short temporal scale (41-54 days) during cool water temperatures (4-20℃), we assumed that predator growth rates were negligible, providing a conservative estimate of predator consumption. Our assumption regarding negligible predator growth rates was also based on published literature. Specifically, Quist et al. (2002) in Kansas reported that across a four year study there was only one year where mean Walleye mass significantly increased between fall (September to November) and winter (January to February), indicating little growth (0-250 g). Additionally, Northern Pike growth from September to November was reported to be near zero in two southern Ohio impoundments (Headrick and Carline 1993) and Northern Pike in Lake Thompson, South Dakota gained 10-30 g from September to November (males: 10-15 g; females: 10-30 g; Neuman et al. 1994). For Largemouth Bass, negligible growth (≤ 6% of maximum somatic growth) occurs ≤ 10℃ (McCauley and Kilgor 1990); 39% of the study duration water temperatures were ≤ 10℃. Furthermore, Largemouth Bass specific growth was ≤ 0.01 g/g/ day at water temperatures ≤ 18℃ (Rice et al. 1983), which was the upper limit of water temperatures observed in the current study. Therefore, we assumed negligible growth here.

Determining the proportion stocked age-0 Walleye consumed by predators

To determine what percent of stocked age-0 Walleye was consumed by predators, we determined the amount of wet weight available for consumption for each system each year. We used wet weight frequency distributions of non-consumed age-0 Walleye for each year and system based on the subset of individuals that were directly weighed before stocking to determine the proportions of age-0 Walleye in 10 g weight bins (Figure 5.1). To determine the

total amount of age-0 Walleye wet weight available for consumption, we used the following equation:

$$\sum TWW_{bin} = N_{WAE} * WBin_{proportion} * WBin_{weight}$$

where TWW_{bin} is the wet weight of non-consumed age-0 Walleye in individual 10 g weight bins, N_{WAE} is the total number of age-0 Walleye stocked in a given year within a system, $WBin_{proportion}$ is the proportion of age-0 Walleye in a specific 10 g weight bin in a given year and system, and $WBin_{weight}$ is the wet weight (g) of each 10 g weight bin. The total wet weight of stocked age-0 Walleye in a given year and system was calculated as the summation TWW_{bin}.

To determine the total amount (g) of consumed age-0 Walleye by each predator species within a given year and system, the bioenergetics model output (weight of age-0 Walleye consumed by a single predator) was multiplied by Schnabel population estimates (and ± 95% confidence intervals; TC_{WAE}). Total percentage of consumed age-0 Walleye population (C_{WAE}) for each predation was calculated as:

$$C_{WAE} = \frac{TC_{WAE}}{\sum TWW_{bin}} \cdot 100$$

where TC_{WAE} is the estimated total amount of age-0 Walleye wet weight (g) consumed by a predator within a system and year based on the mean and 95% confidence intervals of Schnabel population estimates. To avoid underestimating the total percentage of consumed age-0 Walleye, TWW_{bin} was a summation of wet weights for weight bins in which consumed age-0 Walleye were recovered from predator stomachs. We did not subclassify predators into size classes because predator size is not related to the size of age-0 Walleye consumed in these systems (Grausgruber and Weber *in press*)

Determining the proportion differently sized age-0 Walleye consumed by predators

To determine the wet weight of consumed age-0 Walleye by predation within a 10 g weight bin ($WW_{consumed}$), we used the following equation

$$WW_{consumed} = TC_{WAE} * CBin_{proportion}$$

where TC_{WAE} is the total amount of age-0 Walleye wet weight (g) consumed by a predator within a system during each year and $CBin_{proportion}$ is the proportion of consumed age-0 Walleye in a given 10 g weight bin that was specific to year and system. To determine the percentage of consumed age-0 Walleye by a predator within a 10 g weight bin ($\%_{WBin}$) within a given system and year we used the following equation:

$$\%_{WBin} = \frac{WW_{consumed}}{TWW_{bin}} \cdot 100$$

where $WW_{consumed}$ is the wet weight of consumed age-0 Walleye by predation within a 10 g weight bin and TWW_{bin} is the amount of age-0 Walleye wet weight available for consumption in the same 10 g weight bin.

Results

Predator populations

Across all years and systems, 3,272 predators were collected (1,699 Largemouth Bass, 731 Northern Pike, and 842 adult Walleye). The number of predators captured and recaptured varied across species, systems, and years (Table 5.3). In East Okoboji, average recapture rate was 9.74% (SD = 1.50) for Largemouth Bass, 5.17% (SD = 4.20) for Northern Pike, and 6.27% (SD = 0.12) for adult Walleye. In West Okoboji, average recapture rate was 13.01% (SD = 3.79) for Largemouth Bass, 8.83% (SD = 1.41) for Northern Pike, and 13.41% (SD = 5.07) for adult Walleye. Predator population densities varied between 1.8 and 10.3 fish/ha across years and systems (Table 5.3). During 2016 in East Okoboji, Northern Pike had the highest density

followed by Largemouth Bass and adult Walleye (Table 5.3). During 2016 in West Okoboji, Largemouth Bass had the highest density, followed by adult Walleye and Northern Pike (Table 5.3). During 2017 in East and West Okoboji, Largemouth Bass had the highest density followed by adult Walleye and Northern Pike (Table 5.3).

Consumption of prey items other than age-0 Walleye

Predators consumed a variety of prey species, including invertebrates, amphibians, and fishes (Table 5.2). Across sample years, 57% of predators had stomachs that contained at least one prey item. By wet weight, prey categories that comprised the greatest proportion of individual predator diets included fishes in the orders of Centrarchidae, Moronidae, and Sciaenidae (Figure 5.2) whereas prey categories that made up the smallest proportion of predator diets included fishes in the orders of Cyprinidae and Ictaleuridae, as well as terrestrial and aquatic insects (Appendix A). Across all prey categories, prey composition in predator diets depended on interactions between predator and system (LTR = 133.9; $P = 0.001$), predator and year (LTR = 75.2; $P = 0.001$), and system and year (LTR = 47.2; $P = 0.001$). For example, counts of consumed Centrarchidae were dependent on an interaction between predator and system (LTR = 29.14; $P = 0.001$), predator and year (LTR = 32.08; $P = 0.001$), but not system and year (LTR = 0.23; $P = 0.93$; Figure 5.2). Counts of consumed Moronidae varied across predator species (LTR = 44.12; $P = 0.001$), system (LTR = 102.77; $P = 0.001$; Figure 5.2), and year (LTR = 20.89; $P = 0.001$), but there was no evidence of an interaction between all three predictor variables (Figure 5.2). Similarly, counts of consumed Sciaenidae varied across predator species (LTR = 30.47; $P = 0.001$), system (LTR = 24.79; $P = 0.001$), and year (LTR = 52.24; $P = 0.001$) with little evidence of interactions between predictor variables ($P > 0.05$).

Consumption of age-0 Walleye

No age-0 Walleye were recovered from predator stomachs during the 2-week pre-stocking sampling events prior to September age-0 Walleye stocking dates in either lake during both years. Post-stocking, we recovered 257 age-0 Walleye from predator stomachs (2016: 135 age-0 Walleye; 2017: 122 age-0 Walleye) and were able to estimate the wet weight at the time of consumption for 131 Walleye (51%). Overall, Northern Pike (0.12 ± 0.32 age-0 Walleye per individual) had the highest proportion (± SD) of age-0 Walleye in their diets followed by Largemouth Bass (0.11 ± 0.30 age-0 Walleye per individual) and adult Walleye (0.04 ± 0.20 age-0 Walleye per individual). There was little annual variation in the average proportion (± SD) of age-0 Walleye in diets of Northern Pike (2016: 0.13 ± 0.32; 2017: 0.12 ± 0.3), Largemouth Bass (2016: 0.09 ± 0.28; 2017: 0.11 ± 0.31), and adult Walleye (2016: 0.04 ± 0.19; 2017: 0.05 ± 0.22). However, the average proportion (± SD) of age-0 Walleye in the diets of Largemouth Bass (East: 0.03 ± 0.19; West: 0.15 ± 0.35) and adult Walleye (East: 0.003 ± 0.10; West: 0.06 ± 0.30) varied across systems, but Northern Pike (East: 0.11 ± 0.30; West: 0.15 ± 0.34) in both systems had a similar average proportion of age-0 Walleye. Generally, age-0 Walleye made up a greater average proportion (± SD) of predator diets in West Okoboji (0.14 ± 0.33) than East Okoboji (0.05 ± 0.21). Stocked and consumed age-0 Walleye had left-skewed weight distributions that generally consisted of smaller individuals (< 100 g; < 229 mm; Figure 5.1).

Daily proportions of age-0 Walleye in predator diets varied across predators, systems, and years (Figure 5.3). Collectively, the highest proportions of age-0 Walleye in the diets of Largemouth Bass, Northern Pike, and adult Walleye occurred from 1-14 days after the most recent stocking event (Figure 5.3). Typically, 14 days after the first stocking event, the age-0 Walleye were no longer detected in predator diets or the proportion of age-0 Walleye in predator

diets was decreasing (Figure 5.3); however, in 2017, Northern Pike in East Okoboji (days: 15-35) and West Okoboji (days: 14-21) and adult Walleye in West Okoboji (days: 14-21) had increases in the proportion of age-0 Walleye in their diets between the two stocking events (Figure 5.3).

The total percentage of stocked age-0 Walleye consumed by predators varied across systems (Figure 5.4). In 2016, Largemouth Bass, Northern Pike, and adult Walleye in East Okoboji consumed 2.4% (95% CI: 1.0 %, 3.9%) of stocked age-0 Walleye while in 2017 they consumed 9.0% (95% CI: 5.8%, 12.3%). In West Okoboji, Largemouth Bass, Northern Pike, and adult Walleye collectively consumed 15.7% (95% CI: 9.2%, 22.1%) of stocked age-0 Walleye in 2016 and 21.3% (CI: 15.4%, 27.2%) of stocked age-0 Walleye in 2017. By species, Largemouth Bass consumed 0.6% (95% CI: 0.4%, 0.8%) of age-0 Walleye stocked in East Okoboji and 7.2% (95% CI: 4.9%, 9.4%) in West Okoboji during fall 2016 (Figure 5.4) whereas during fall 2017, Largemouth Bass consumed 1.9% (95% CI: 1.3%, 2.5%) of age-0 Walleye stocked in East Okoboji and 11.7% (95% CI: 9.5%, 13.9%) stocked in West Okoboji (Figure 5.4). During fall 2016, Northern Pike consumed 1.8% (95% CI: 0.5%, 3.0%) of stocked age-0 Walleye in East Okoboji and 7.0% (95% CI: 3.4%, 10.5%) in West Okoboji (Figure 5.4) whereas during fall 2017, Northern Pike consumed 6.2% (95% CI: 4.0%, 8.4%) of stocked age-0 Walleye in East Okoboji and 6.9% (95% CI: 4.1%, 9.6%) in West Okoboji (Figure 5.4). Adult Walleye were estimated to consume a small percentage of stocked age-0 Walleye in East Okoboji (0-1.4%) and West Okoboji (0.8-3.7%; Figure 5.4).

Collectively, predators in West Okoboji consumed higher percentages (28-282%) of smaller (10-89g; TL: 64-221mm) age-0 Walleye relative to predators in East Okoboji (5-120%; Figure 5.5). Across years and systems, adult Walleye were estimated to consume <1% of age-0

Walleye at any weight classification. Largemouth Bass, Northern Pike, or adult Walleye did not consume significant percentages (>10%) of age-0 Walleye that were ≥ 90 g (TL: ≥ 222 mm; Figure 5.5). During 2017 in West Okoboji, Largemouth Bass and Northern Pike were estimated to consume all of the 20-29 g (TL: 114-140 mm) age-0 Walleye and 33% (95% CI: 19-46%) of the 20-29 g (TL: 114-140 mm) age-0 Walleye during 2016. In East Okoboji during 2017, Largemouth Bass and Northern Pike collectively consumed 57% (95% CI: 37-76%) of age-0 Walleye that were 30-39 g (TL: 143-162 mm) and 13% (95% CI: 5-21%) in 2016. Across both systems and years, Largemouth Bass and Northern Pike consumed 9% (95% CI: 6-12%) of each 10 g weight bin ranging between 30-89 g (TL: 143-221 mm).

Discussion

A reoccurring conclusion drawn from post-stocking evaluations is the potential for local piscivores to negatively influence stocked fishes through high post-stocking consumption (Stein 1981; Szendrey and Wahl 1996; Diana and Wahl 2009; Freedman et al. 2012). Largemouth Bass (Santucci and Wahl 1993; Fayram et al. 2005; Freedman et al. 2012), Northern Pike (Anthony and Jorgensen 1977; Liao et al. 2002; Nate et al. 2003), and adult Walleye (Liao et al. 2002) have been observed to consume age-0 Walleye at various sizes and life stages (fry and fingerling). As hypothesized, consumption of age-0 Walleye varied across predators, systems, and years, with Northern Pike and Largemouth Bass consuming more age-0 Walleye and having higher proportions of age-0 Walleye in their diets compared to adult Walleye. Largemouth Bass have been previously documented to consume 0-23% of stocked age-0 Walleye (Santucci and Wahl 1993; Freedman et al. 2012) while Northern Pike have been documented to consume up to 49% of stocked age-0 Walleye (< 229 mm; Johnson et al. 1996). Although cannibalism has been observed to negatively influence stocked Walleye populations (Forney 1976; Li et al. 1996), we

observed that adult Walleye consumed a small percentage of age-0 Walleye in this study (< 4% biomass) compared to other piscivores.

Weber et al. (2020) estimated 25% cumulative mortality of stocked Walleye (233-237 mm) across three Iowa lakes, one of which included East Okoboji, with many of the mortalities in this system attributed to predation. Based on our results, Largemouth Bass, Northern Pike, and adult Walleye can collectively consume 2-9% of stocked age-0 Walleye during the fall (8-36% of the mortality). If age-0 Walleye survival was the same in West Okoboji, and predators consumed 16-21% of the stocked age-0 Walleye population, then post-stocking predation would account for 64-84% of mortality. Thus, post-stocking predation has the potential to be a significant limiting factor that influences cumulative post-stocking mortality.

Predation is a size-structured interaction where smaller members of cohorts are routinely the most vulnerable to predation (Chevalier 1973; Wahl and Stein 1989; Weber et al. 2011). Larger body size at stocking has generally been linked to reduced predation risk (Gaeta et al. 2018; Grausgruber and Weber *in press*) and higher survival for a number of species (e.g., Laarman 1978; Santucci and Wahl 1993; Fayram et al. 2005; Diana and Wahl 2009). Thus, we initially hypothesized that smaller age-0 Walleye would be preyed upon at higher rates relative to larger conspecifics. Our results supported our hypothesis, where Largemouth Bass and Northern Pike were estimated to consume between 33-100% of the stocked age-0 Walleye 20-89 g (114-221 mm), suggesting that fisheries managers should consider stocking age-0 Walleye ≥ 90 g (TL ≥ 220 mm) in situations where post-stocking predation is a concern. However, prior evaluations have reported smaller sizes of age-0 Walleye that are predated upon less frequently and have higher survival rates. For example, Santucci and Wahl (1993) reported that larger fingerling Walleye (186-216 mm) had higher survival rates than small (48-61 mm) and medium (132-145

mm) age-0 Walleye when stocked into a centrarchid (Largemouth Bass) dominated impoundment. Alternatively, Brooks et al. (2002) and Koppelman et al. (1992) reported that smaller fingerling Walleye (50 mm and 25-51 mm, respectively) had higher survival rates than larger fingerling Walleye (91-122 mm). Variability across studies could in part be due to the size of age-0 Walleye stocked as well as the abundance and sizes of predators. Lawson and Carpenter (2014) developed a morphometric relationship that determined what size of age-0 Walleye (10-220 mm) are outside the gape limitations of Rainbow Smelt *Osmerus mordax*. However, Grausgruber and Weber (*in press*) found little evidence that predation on large age-0 Walleye (95-253 mm) was related to gape height or total length of Largemouth Bass, Northern Pike, Smallmouth Bass, or adult Walleye. To date, there has not been a clear general recommendation of how large stocked age-0 Walleye need to be to escape the gape limitations of a suite of piscivores. Our results suggest piscivores of various sizes rarely consume Walleye ≥ 90g (TL = 220 mm) and that stocking age-0 Walleye greater than 220 mm may enhance post-stocking survival and improve stocking success.

Generally, predation on stocked fish is highest soon after stocking (12- 24 hours post-stocking; Buckmeier et al. 2005; Freedman et al. 2012). We observed a majority of age-0 Walleye in diets of Northern Pike, Largemouth Bass, and adult Walleye up to 28 days after the most recent fall stocking event. However, we were limited by our ability to intensively sample for an extended period (up to 54 days) following the second stocking event when the lake was ice-covered. Thus, there is the potential that the consumption of age-0 Walleye could have occurred throughout the winter under the ice when we were unable to sample.

Stocking is an important management tool for maintaining recreational fisheries and for replenishing declining populations of Walleye. However, hatchery production is an expensive

process that is dependent on post-stocking survival. Based on our bioenergetics estimates (mean ± 95% CI) and average production costs (cost associated with feed, medication, personnel, etc.) an advanced fingerling age-0 Walleye ($1.32 ± 0.44 SD; C. Clouse, Iowa DNR, personal communication), Northern Pike and Largemouth Bass in East Okoboji and West Okoboji collectively consumed $2,478 (95% CI: $1,350; $3,608) of stocked age-0 Walleye in 2016 and $6,064 (95% CI: $4,277; $7,845) in 2017 within 54 days post-stocking. Thus, from an economic standpoint, fall post-stocking predation can have financial ramifications. Stocking additional age-0 Walleye could offset losses due to post-stocking predation or stocking age-0 Walleye at sizes rarely consumed by predators (≥ 90g; TL = 220 mm) could result in improved post-stocking survival by decreasing the number of age-0 Walleye consumed by predators: both of these options would result in added hatchery production costs. Stocked fishes are occasionally placed in acclimation cages for 3-6 days to decrease post-stocking predation (Jonsson et al. 1999; Brennan et al. 2006) but would not likely be beneficial in situations where post-stocking predation is prolonged as observed by predators in West Okoboji. Alternatively, predator recognition training during the rearing process can condition fish to recognize chemical and visual cues of predators has shown promise in decreasing post-stocking predation in salmonid species (Brown and Smith 1998; Brown and Laland 2001); however, mass-training on percid species has been less successful (Wisenden et al. 2004). Ultimately, post-stocking predation has the potential to remove a significant portion of a stocked age-0 Walleye population and developing new rearing, and stocking techniques to decrease predation may offer a methodology for improving age-0 Walleye stocking success.

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Tables

Table 5.1 Estimated number of age-0 Walleye stocked based on weight estimations in East Okoboji and West Okoboji, Iowa, USA during four dates in 2016 and 2017. The bioenergetic day is the day number in which the stocking date was included in bioenergetics modeling.

Date	Bioenergetic day	Number in West Okoboji	Number in East Okoboji
2016			
September 27	1	9,616	-
September 28	1	-	9,621
October 31	35	2,345	1,556
November 1	36	2,243	3,029
Total		14,204	14,206
2017			
September 28	1	9,627	9,632
October 23	26	1,000	1,029
October 30	33	-	3,589
November 1	35	5,584	-
Total		16,211	14,250

Table 5.2 Prey species present in the stomach of Largemouth Bass (LMB), Northern Pike (NOP), and Walleye (WAE). Prey species were separated into prey groups (age-0 Walleye, anuran, Centrarchidae, Clupeidae, Cyprinidae, Decapoda, Ictaleuridae, Insecta, Moronidae, other fish, Percidae, Sciaenidae, and unidentifiable) that were incorporated into bioenergetics modeling. Organisms with an energy density value (J/g wet weight) denote prey items used in the bioenergetics modeling.

Prey Group	Prey Species	Energy density (J/g wet weight)	LMB	NOP	WAE
Age-0 Walleye		4,557			
	Walleye *Sander vitreus*		X	X	X
Anuran (Adult)		1,700			
	American Bullfrog *Rana catesbeiana*		X	X	
	Northern Leopard Frog *Rana pipiens*		X	X	X
Anuran (Larval)		290			
	Bullfrog tadpole *Rana catesbeiana*		X	X	X
Centrarchidae		1,160			
	Black Crappie *Pomoxis nigromaculatus*		X		X
	Bluegill *Lepomis macrochirus*		X	X	X
	Green Sunfish *Lepomis cyanellus*		X	X	X
	Largemouth Bass *Micropterus salmoides*		X	X	X
	White Crappie *Pomoxis annularis*		X	X	X
Clupeidae		5,108			
	Gizzard Shad *Dorosoma cepedianum*			X	X
Cyprinidae		5,996			
	Common Carp *Cyprinus carpio*		X	X	X
	Golden Shiner *Notemigonus crysoleucas*		X		
	Spottail Shiner *Notropis hudsonius*			X	
Decapoda		3,700			
	Northern Crayfish *Oronectes virilis*		X		X
Ictaleuridae		4,557			
	Black Bullhead *Ameiurus melas*		X		
	Channel Catfish *Ictalurus punctatus*		X		X
	Yellow Bullhead *Ameiurus natalis*			X	
Insecta		3,758	X		X
Moronidae		6,222			
	Yellow Bass *Morone mississippiensis*		X	X	X
Other Fish		4,688			
	Northern Pike *Esox lucius*			X	
	Unidentifiable Fish		X	X	X

Prey Group	Prey Species	Energy density (J/g wet weight)	LMB	NOP	WAE
Percidae		4,186			
	Johnny Darter *Etheostoma nigrum*				X
	Log Perch *Percina caprodes*			X	
	Yellow Perch *Perca flavescens*		X	X	X
Sciaenidae		5,786			
	Freshwater Drum *Aplodinotus grunniens*		X	X	X
Unidentifiable		3,977	X	X	X

Table 5.3 The number of captures, recaptures, percent recaptures, average individual weight (g), Schnabel population estimates ($\widehat{N}$; ±95% CI), and densities (individuals/ha) of Largemouth Bass, Northern Pike, and adult in East Okoboji and West Okoboji, Iowa, USA during fall 2016 and 2017.

Year & System	Number Captured	Number Recaptured	Percent Recapture	Weight (g)	$\widehat{N}$	95% CI		Individuals /ha
2016 East Okoboji								
Largemouth Bass	379	36	8.67	820	1,568	1,076	2,060	6.8
Northern Pike	133	3	2.21	1,238	729	222	1,236	8.6
Walleye	379	25	6.19	582	1,970	1,265	2,676	2.0
2016 West Okoboji								
Largemouth Bass	295	34	10.33	955	971	670	1,271	6.4
Northern Pike	106	9	7.82	955	321	158	483	1.8
Walleye	101	11	9.82	846	259	136	382	4.9
2017 East Okoboji								
Largemouth Bass	289	35	10.80	839	921	636	1,207	4.0
Northern Pike	282	25	8.14	1,325	1,102	714	1,490	2.6
Walleye	162	11	6.35	677	605	308	903	3.2
2017 West Okoboji								
Largemouth Bass	532	99	15.69	1,033	1,571	1,272	1,870	10.3
Northern Pike	156	17	9.83	1,096	472	283	661	2.0
Walleye	127	26	16.99	1,063	305	199	410	2.1

Figures

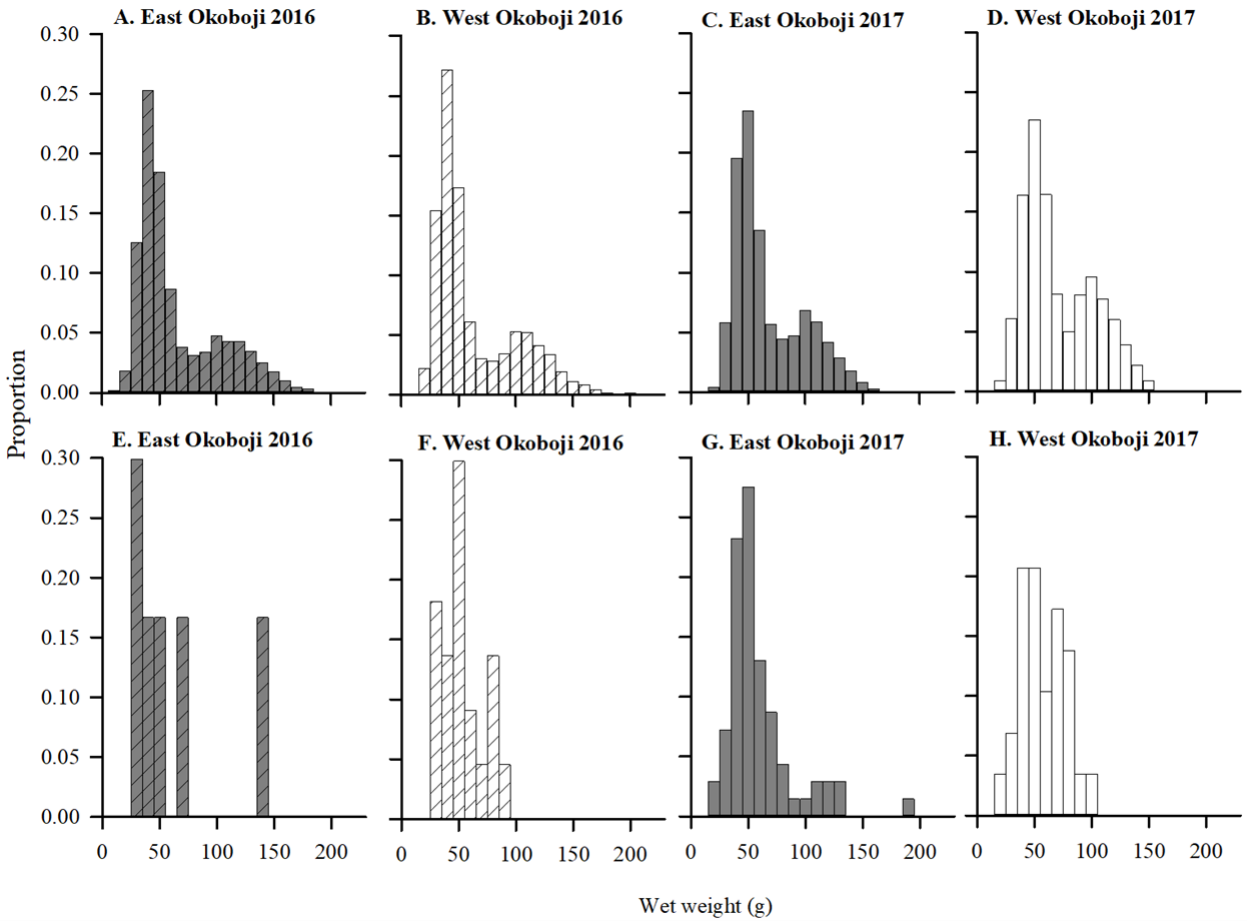

Figure 5.1 Wet weight-frequency histogram of Walleye in 10 g weight bins across sample years (2016 = diagonal lines; 2017 = solid fill) and systems (East Okoboji = dark grey; West Okoboji = light grey). The top row (A-D) denotes the proportion stocked age-0 Walleye while the bottom row denotes the proportion of consumed age-0 Walleye (E-H).

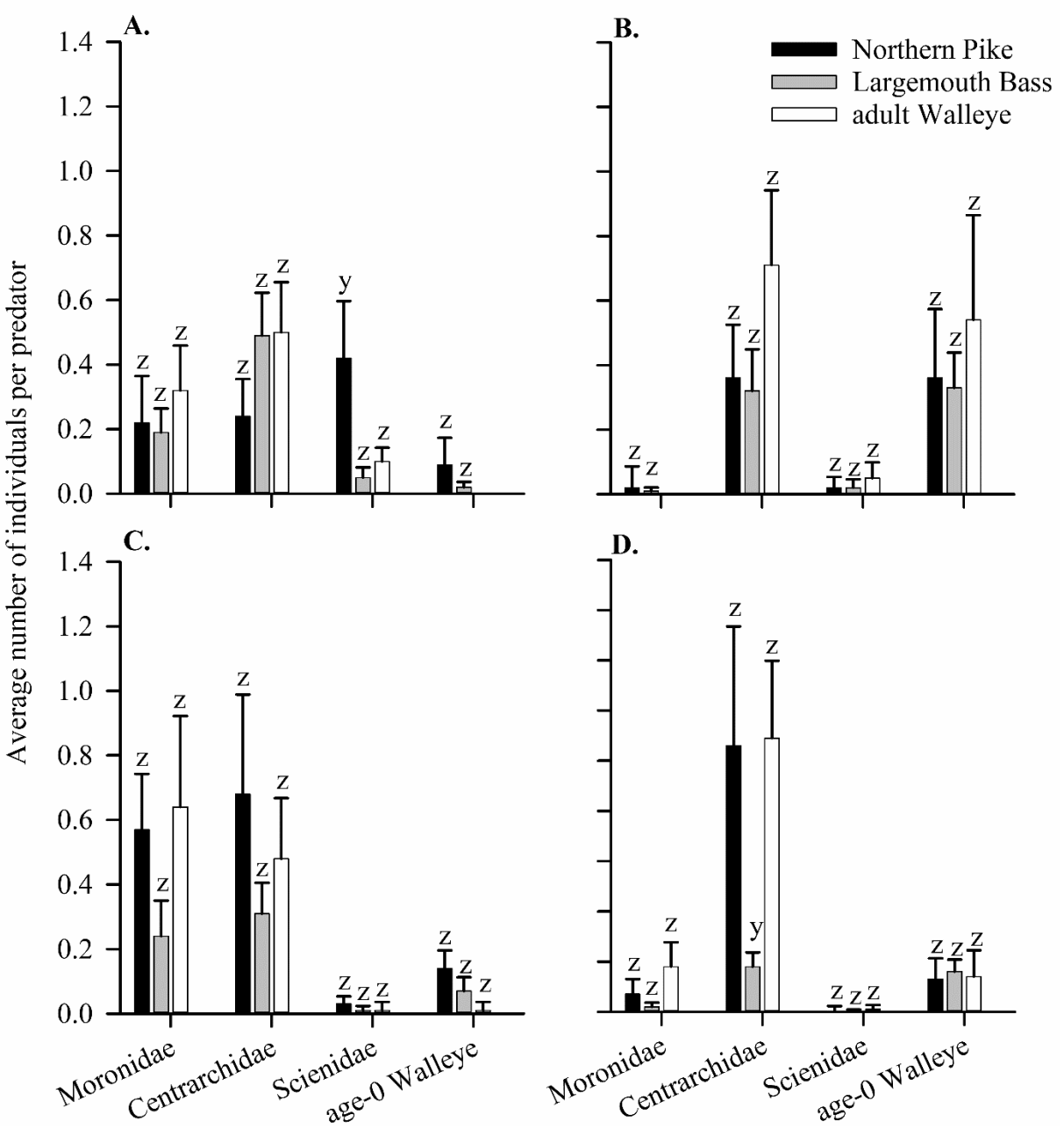

Figure 5.2 Average (± 95% CI) number of Moronidae, Centrarchidae , Sciaenidae, and age-0 Walleye recovered from Largemouth Bass (grey), Northern Pike (black), and adult Walleye (white) from East Okoboji (A&C) and West Okoboji (B&D) during 2016 (A&B) and 2017 (C&D). Bars that share similar letters within a specific panel and prey species are not significantly different.

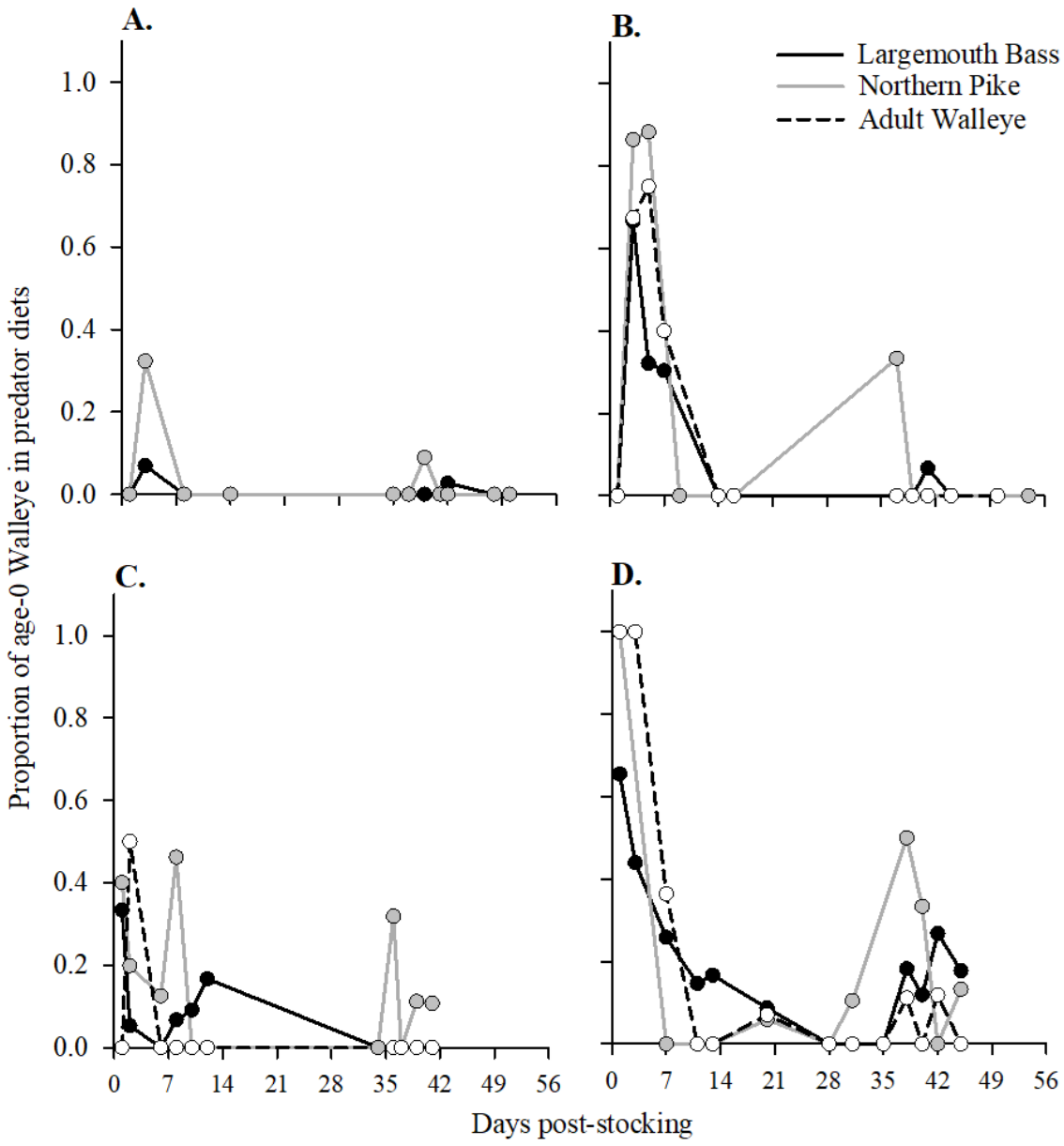

Figure 5.3 Daily proportion of stocked age-0 Walleye in predator (Largemouth Bass = solid black line, Northern Pike = solid grey line, and adult Walleye = dashed black line) diets in East Okoboji (A & C) and West Okoboji (B & D) in 2016 (A & B) and 2017 (C & D) based on bioenergetics modeling (lines) and points indicate dates in which sampling occurred. The second increase in values corresponds with the second stocking event on day 35 in 2016 and day 37 in 2017.

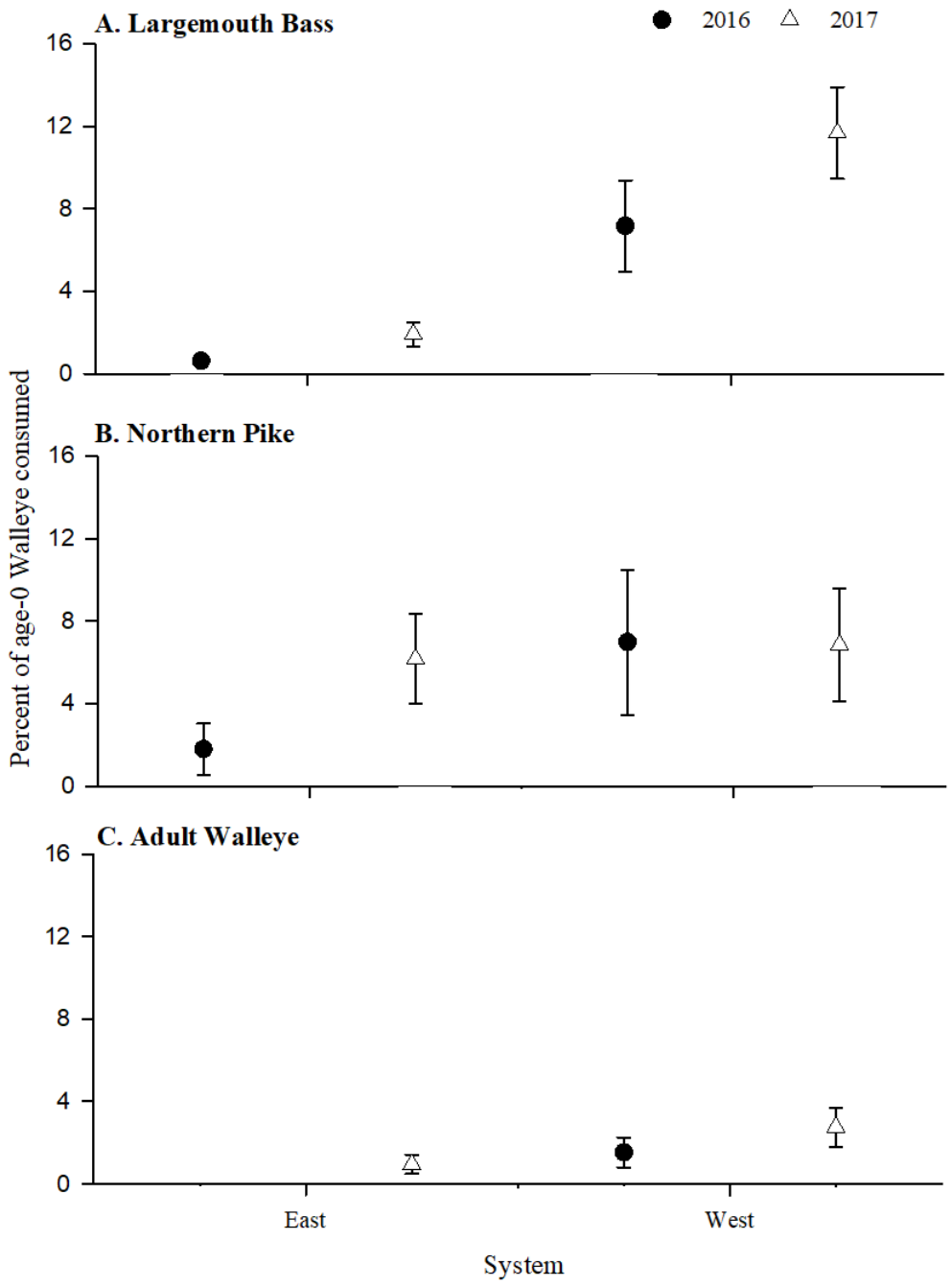

Figure 5.4 Total percent of stocked age-0 Walleye consumed by Largemouth Bass (A), Northern Pike (B), and adult Walleye (C) in East Okoboji and West Okoboji during 2016 (black circle) and 2017 (white triangle) based on mean and 95% confidence interval predator population estimates.

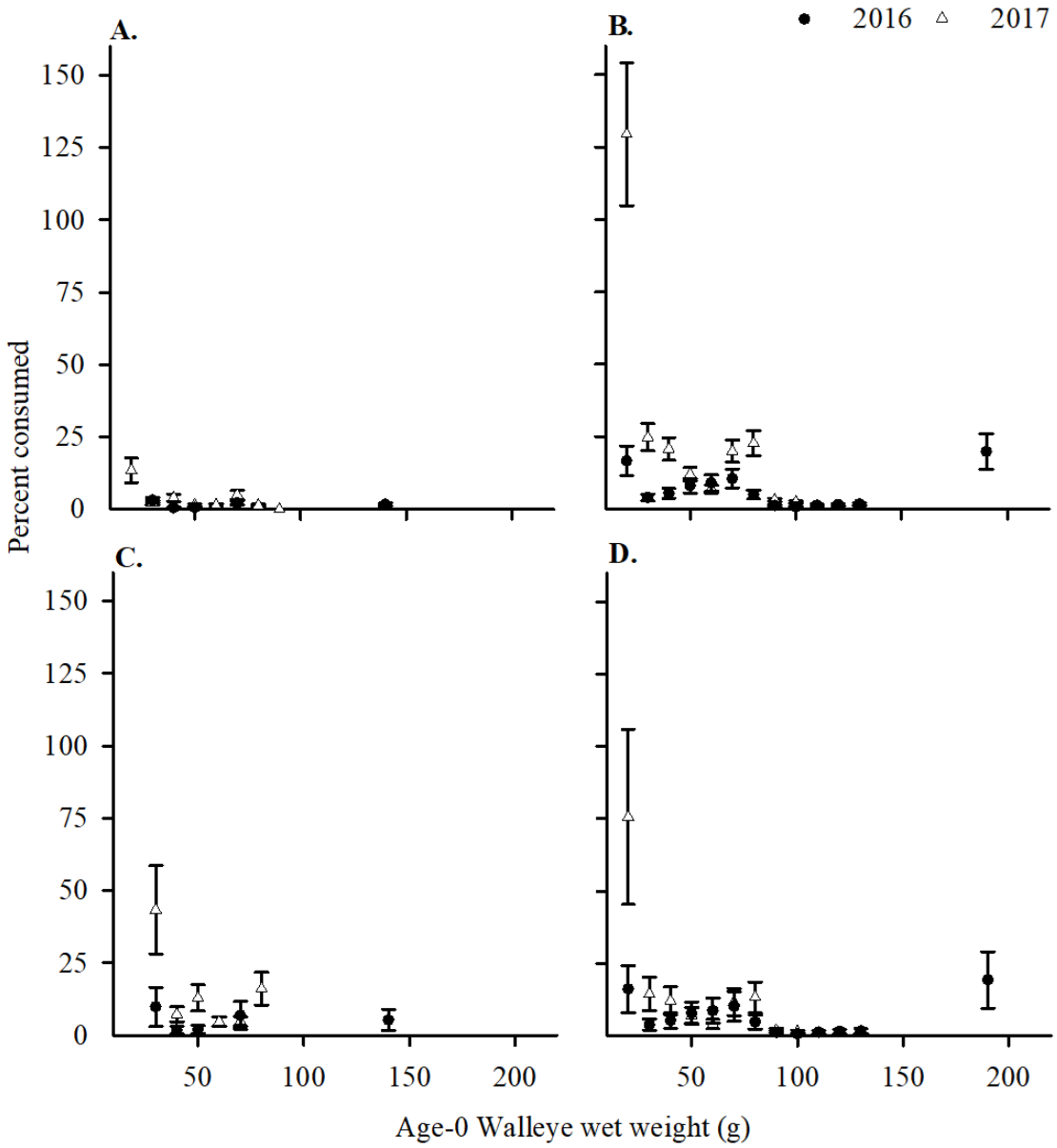

Figure 5.5 Size-selective consumption of stocked age-0 Walleye (10 g weight bin) by Largemouth Bass (A & B) and Northern Pike (C & D) in East Okoboji (A & C) and West Okoboji (B & D) in 2016 (black circle) and 2017 (white triangle). Vertical lines denote consumption percentages based on high and low density of predators while symbols denote the median predator density.

CHAPTER 6. SHIFT HAPPENS: EVALUATING THE ABILITY OF FALL STOCKED WALLEYE *SANDER VITREUS* TO SHIFT TO NATURAL PREY

Modified from a manuscript to be submitted to Fisheries Management and Ecology

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Abstract

Hatchery propagation techniques, such as pellet-rearing, can result in impaired post-stocking feeding behavior of fishes. Walleye *Sander vitreus* is a commonly propagated sportfish; yet, little is known regarding whether hatchery propagation techniques (pellet-rearing) influence post-stocking diet composition. Our objective was to compare fall diets of age-0 wild and stocked Walleye by evaluating the proportion of empty stomachs as well as proportions of zooplankton, benthic invertebrates, and fish in diets. We used percent similarity index values to assess the percent similarity of wild and stocked Walleye diets. During 2016 and 2017, Walleye were stocked in late September, nightly boat electrofishing was used to capture Walleye, and pulsed gastric lavage was used to collect stomach contents. In total, 590 Walleye were gastrically lavaged, with wild Walleye making up 9.7% and stocked Walleye making up 90.3% of the samples. The average proportion of empty stomachs differed between wild and stocked Walleye, with stocked Walleye having higher average (± 95% CI) proportions of empty stomachs (0.40 ± 0.10) compared to wild Walleye (0.15 ± 0.09). Proportion of empty stomachs and proportion of Walleye with zooplankton in diets decreased with days post-stocking. Stocked Walleye had a greater proportion of benthic invertebrates whereas wild Walleye consumed more fish. However, Walleye total length was not related to the presence of zooplankton, benthic invertebrates, or fish in wild (TL = 129-215 mm) and stocked (TL = 94-228 mm) Walleye diets. Percent similarity

index values for wild and stocked Walleye diets were highly variable and ranged from 0.0 to 67.9%. Collectively, our results suggest that fall stocked Walleye consume different prey items compared to their wild counterparts for up to 49 days post-stocking that may have implications for post-stocking survival.

Introduction

Propagated hatchery fishes are often used as management tools for supplementing populations with low recruitment (Nickum, Mazik, Nickum, & MacKinlay, 2004; Wedemeyer, 2001). The success of stocking programs is ultimately dependent on post-stocking survival and can be influenced by a large number of factors including body size, predation, and transportation practices (Ball, Goode, & Weber, 2020; Forsberg, Summerfelt, & Barton, 1999; Litvak & Leggett, 1992; Meekan, Vigliola, Hansen, Doherty, Halford, & Carleton, 2006; Santucci & Wahl, 1993). Additionally, post-stocking foraging behavior and malnutrition may also be an important determinant for fish survival (Kellison, Eggleston, & Burke, 2000; Szendrey & Wahl, 1996), but has received considerably less attention compared to the aforementioned factors.

Since the 1980s, there has been a growing concern regarding hatchery habituation and whether hatchery fish lack natural foraging behaviors (Johnsen & Ugedal, 1989; Sosiak, Randall, & McKenzie, 1979; Sundstom & Johnsson, 2001) due to rearing practices and habituation to consuming pellets rather than actively foraging on live prey. Studies evaluating post-stocking feeding behaviors of hatchery-reared fish have had varying results that differ across species. For instance, pellet-rearing has been reported to not influence feeding behaviors of Coho Salmon *Oncorhynchus kisutch* (Paszkowski & Olla, 1985) whereas pellet-reared Walleye *Sander vitreus* (Wahl, Einfalt, & Hooe, 1995), Muskellunge *Esox masquinongy* (Larscheid, Christianson, Gengerke, & Jorgensen, 1999), and Atlantic Salmon *Salmo salar* (Brown, Davidson, & Laland, 2003; Johnson, McKeon, & Dropkin, 1996) exhibit slow conversion to live prey items, higher

percentages of empty stomachs, and lower post-stocking survival rates. Prior research suggests that pellet rearing can negatively influence stocking success. However, it is not clear how long feeding behaviors of pellet reared fish remained altered or whether fish size influences post-stocking diets of pellet reared fish. Understanding whether pellet reared hatchery fish have innate responses associated with feeding on live prey will provide evidence as to whether post-stocking starvation could be a bottleneck limiting stocking success.

Walleye is a popular sportfish throughout North America and is often stocked to supplement and maintain populations (Barton, 2011; Ellison & Franzin, 1992; Fenton, Mathais, & Moodie, 1996). Walleye undergo ontogenetic shifts during their first year that are critical for growth and survival (Einfalt & Wahl, 1997; Mathais & Li, 1982; Pelham, Pierce, & Larscheid, 2001; Quist, Guy, Bernot, & Stephen, 2002; Raisanen & Applegate, 1983; Uphoff, Shoenebeck, Koupal, Pope, & Hoback, 2019). Walleye begin feeding on zooplankton after endogenous feeding at about 8 mm total length (TL) before switching to macroinvertebrates (35-50 mm TL) and eventually to fish (60-80 mm TL; Galarowicz & Wahl, 2005; Mathias & Li, 1982; Priegel, 1969; Uphoff et al., 2019). Failure of Walleye and other fishes to undergo diet shifts can result in reduced energy reserves, growth, and overwinter survival (reviewed by Olla, Davis, & Ryer, 1998). There is a vast amount of literature on Walleye diet composition and ontogeny (Galarowicz & Wahl, 2005; Hoxmeier, Wahl, Brooks, & Heidinger, 2006; Jackson, Willis, & Fielder, 1992; Mathias & Li, 1982; Priegel, 1969; Ward, Willis, Herwig, Chipps, Parsons, Reed, & Hanson, 2008; Uphoff et al., 2019). However, less is known regarding the influence of pellet rearing on Walleye feeding behavior and diet composition. In laboratory settings, Walleye with previous experience foraging on minnows had quicker conversion to live forage, decreased capture times, and consumed more live prey relative to pellet reared Walleye (Wahl, Einfalt, &

Hooe, 1995). Thus, ontogeny and diet composition of Walleye may be dependent on experience and effectiveness at capturing live prey items. If pellet reared Walleye do have altered feeding behavior, they may initially lack behaviors associated with locating and capturing live, mobile prey and rely on less mobile, less nutritious prey.

Walleye are often stocked as either fry (9 mm) or fingerlings (78-295 mm). Stocked Walleye are reared on pellet diets for three to four months before fall stocking from mid-September to the beginning of November. Stocking progressively larger Walleye is done with the intention of circumventing processes that negatively influence the recruitment of young-of-year fish, including size-selective predation (Grausgruber & Weber, *in press*) and starvation (Miller, Crowder, Rice, & Marschall, 1988). Increased size at the time of stocking is thought to decrease predation risk (Miranda & Hubbard, 1994; Post & Evans, 1989) due to improved maneuverability and swimming speed (Videler, 1993). Furthermore, larger body sizes can be associated with decreased starvation through increased gape size which allows for consumption of larger more nutritious prey (fish). However, intensive propagation of stocked Walleye requires prolonged rearing duration and an intense feeding regime, which has the potential to result in habituation and loss of natural feeding behaviors necessary for capturing live prey (Wahl, Einfalt, & Hooe, 1995). The contribution of stocked Walleye to the adult populations can be low (~10%; McWilliams & Larscheid, 1992; Pratt & Fox, 2003), possibly due to low survival rates (Weber, Weber, Ball, & Meerbeek, 2020; Weber & Weber, *in press*). If recently stocked Walleye are unable to consume live prey immediately after stocking, or if they are limited to consuming less energetically profitable food sources (e.g., zooplankton or benthic invertebrates), they could experience increased starvation, lower growth, and higher predation, resulting in decreased stocking success.

Our objective was to compare diets of fall stocked fingerling versus wild Walleye. We hypothesized that wild Walleye would have lower proportions of empty stomachs, higher proportions of fish, and lower proportions of zooplankton and benthic invertebrates in their diets relative to stocked Walleye. Additionally, we hypothesized that the proportion of stocked Walleye with empty stomachs would decrease, whereas the proportions of benthic invertebrates and fish in stocked Walleye diets would increase through time. Finally, we hypothesized that larger stocked Walleye would consume larger prey items (i.e., fish). Collectively, our results will provide insights regarding the ability of pellet reared stocked Walleye to transition to and successfully foraging on live prey.

Methods

Study systems

East Okoboji and West Okoboji lakes are located in Dickinson County, Iowa. East Okoboji is a long, narrow, and shallow eutrophic natural lake (743 ha) with a 4,767-ha watershed that consists primarily of agriculture. The basin of East Okoboji slopes gradually and has a mean depth of 3.2 m and a maximum depth of 6.7 m. West Okoboji is a large oligotrophic natural lake (1,557 ha) with a 6,133-ha watershed that also consists primarily of agriculture. Relative to East Okoboji, West Okoboji has a more complex basin characterized by sharp drop-offs, numerous rock reefs, and extensive rock-rubble substrates with a mean depth of 11.6 m and a maximum depth of 40.8 m. East Okoboji and West Okoboji are interconnected via a canal located on the southeast corner of West Okoboji. Submersed aquatic vegetation abundant in both East Okoboji and West Okoboji includes Wild Celery *Vallisneria americana,* Flatstem Pondweed *Potamogeton zosteriformis,* Curly-leaf Pondweed *Potamogeton crispus*, Bushy Pondweed *Najas flexillis,* Clasping Leaf Pondweed *Potamogeton richardsonii,* and Coontail *Ceratophyllum demersum*. Curly-leaf Pondweed dominates the upper two basins of East Okoboji from late fall

to early summer. Potential Walleye prey fishes present in both systems include young-of-year Bluegill *Lepomis macrochirus*, Yellow Bass *Morone mississippiensis*, Freshwater Drum *Aplodinotus grunniens*, as well as Johnny Darters *Etheostoma nigrum*, and Spottail Shiners *Notropis hudsonius*.

Fingerling Walleye stocking

East Okoboji and West Okoboji were stocked with fingerling Walleye (98-287 mm) during fall 2016 and 2017. Each year, approximately 19,000 stocked Walleye originating from Spirit Lake Fish Hatchery were stocked across both systems (2016: 19,240 stocked Walleye; 2017: 19,259 stocked Walleye). At the hatchery, stocked Walleye were reared on a pellet diet for five months before stocking (A. Havard, Iowa Department of Natural Resources, *personal communication*). Stocked Walleye were transported between 2.6 and 18.2 km and were stocked in late September (2016: September 27th [East Okoboji] and 28th [West Okoboji]; 2017: September 28th [both systems]). One week before stocking, all Walleye received a pelvic fin-clip for identification from wild Walleye. Fin-clipping is a cost-effective way to mark large numbers of Walleye and does not influence survival or growth rates (Pratt & Fox, 2002).

Post-stocking monitoring

After fingerling stocking events, wild and stocked Walleye were sampled via nighttime boat electrofishing (220V, 5-8 amps pulsed DC; Serns, 1982, 1983) in each system from mid-September and continued until ice-up in mid-November (2016: September 27th to November 16th; 2017: September 28th to November 14th). Natural production of Walleye in East Okoboji and West Okoboji is negligible (J. Meerbeek, Iowa Department of Natural Resources, *personal communication*). Thus, captured Walleye that were not fin clipped are assumed to have originated from spring fry stocking events and were classified as wild Walleye (McWilliams & Larscheid, 1992; J. Meerbeek, IDNR, *unpublished data*). Each system was sampled

approximately every other night for approximately 23 to 24 nights each year (2016: 23 nights; 2017: 24 nights). Weekly average (95% CI) boat electrofishing shock time per system was 9.6 (± 3.7) hr. Due to the large size of the systems and limited post-stocking Walleye movement patterns (Weber et al., 2020), our primary sampling region consisted of a 229-ha area in East Okoboji and a 152-ha area in West Okoboji. Each night boat electrofishing occurred, we sampled the circumference of the 229-ha area in East Okoboji and a 152-ha area in depths ≤ 3.0 m.

Upon capture, Walleye were measured (TL; mm), fin clips were used to identify cohort (wild or stocked), and stomach contents were non-lethally collected via pulsed gastric lavage (PGL) using tap water treated with Tetra AquaSafe (Tetra, Spectrum Brands; Blankman, Ball, & Weber, 2018). Each Walleye underwent triplicate flushing of 140 mL/flush (total of 420 mL of water), and contents from the triplicate flushing were pooled into one Whirl-Pak bag for each Walleye and preserved with 95% ethanol (Blankman, Ball, & Weber, 2018). Stomach contents were concentrated by filtering the sample through a 63-µm-mesh Nitex filter, put into a plankton counting wheel, and identified under a dissecting microscope (Blankman, Ball, & Weber, 2018). Walleye stomachs were classified as being empty or containing at least one prey item. Prey items recovered from Walleye stomachs were identified into taxonomic classifications (Calanoid, Cladoceran, Cyclopoid, Rotifer, Amphipoda, Chironomidae, Coleoptera, Diptera, Ephemeroptera, Hemiptera, Odonata, Trichoptera, Trombidiformes, Centrarchidae, Cyprinidae, Sciaenidae, Hirudinea, and unidentifiable) and then broadly classified as zooplankton, benthic invertebrates, fish, and other for statistical analyses (Table 6.1). Taxonomic and broad classification of stomach contents were reported as mean proportion by number by a given Walleye cohort (wild versus stocked) on a given day. Mean proportion by number was calculated as

$$Mean\ proportion\ by\ number = \frac{1}{P}\sum_{j=1}^{P}\left(\frac{N_{ij}}{\sum_{i=1}^{Q} N_{ij}}\right)$$

where *P* is the number of Walleye in a given cohort (wild 2016, stocked 2016, wild 2017, stocked 2017) with food in their stomachs, *Q* is the number of prey categories, and N_{ij} is the number of prey type *i* in Walleye *j* (Bowen, 1996). Throughout the manuscript proportions of prey items refers to mean proportion by number.

Statistical analysis

In 2016, we compared wild and stocked Walleye prey category proportion data. We used the packages 'vegan' (Oksanen, Guillaume Blanchet, Friendly, Kindt, Legendre, McGlinn, Minchin, O'Hara, Simpson, Solymos, Stevens, Szoecs, & Wagner, 2019) and 'MASS' (Venables & Ripley, 2002) and the function 'metaMDS' in vegan. The packages were used to analyze our data with non-metric multidimensional scaling (NMDS). NMDS is an indirect gradient analysis approach that uses ordination based on a distance dissimilarity matrix (Kruskal, 1964 a,b). NMDS is a rank-based approach; thus, original distance data is substituted with ranks and can tolerate missing pairwise distances (Kruskal, 1964 a,b). We used a Bray-Curtis dissimilarity index to quantify the differences in proportions of prey categories between the two different Walleye cohorts (wild and stocked). We allowed 'initMDS' function in vegan to attempt up to 100 random starts to identify the two most supported convergent solutions. In the current analysis, two convergent solutions were found after 20 random starts with a stress value of 0.09. Stress values equal to or below 0.1 are considered fair, while values equal to or below 0.05 indicate good model fit (Kruskal, 1964 a,b). The vegan package functions 'ordiplot' and 'ordiellipse' were used the develop ellipses on NMDS output plots. Ellipses were used to visually compare diets of wild and stocked Walleye in 2016.

We used a series of linear regression models to evaluate whether proportion of prey categories varied across days post-stocking and cohorts (wild versus stocked). In 2016, we used linear regression models to evaluate the effects of days post-stocking and cohort (wild versus stocked) on the average proportion of empty stomachs and proportions of zooplankton, benthic invertebrates, and fish in wild and stocked Walleye diets. In 2017, we only captured two wild Walleye (East Okoboji = 1; West Okoboji = 1) despite 58.8 electrofishing hours. Thus, for 2017, linear regression models evaluated the effects of days post-stocking on the proportion of stocked Walleye with empty stomachs as well as proportions of zooplankton, benthic invertebrates, and fish in stocked Walleye diets. All linear models were evaluated to ensure that model assumptions regarding the independence of residuals, residuals being normally distributed, and residuals having equal variance were met (McDonald, 2014).

We used a percent similarity index (P) to compare the diet composition of wild and stocked Walleye during 2016. To calculate percent similarity index values, prey species abundance for each prey category was standardized to proportions by dividing the abundance of each prey category in a sample by the total number of prey items in the sample and then multiplying by 100. The similarity, P, between wild and stocked Walleye was calculated as (Krebs, 1999)

$$P_{sw} = \sum_{i} \min(p_{s_i}, p_{w_i})$$

where P is the percent similarity of diet composition between stocked (s) and wild (w) Walleye at sampling event t, p_{fi} is the percent of prey category i in stocked Walleye, and p_{wi} is the percent of prey category i in wild Walleye. Index values range from 0 (no similarity) to 100 (complete similarity). Thus, a value of 50 would indicate that half of the diet contents in wild and stocked

Walleye were similar. This index is not influenced by the number of individuals in a sample and is insensitive to size displacements (Guy & Brown, 2007).

Logistic regressions were used to evaluate whether wild and stocked Walleye total length was related to the presence of empty stomachs and individual prey categories (zooplankton, benthic invertebrates, and fish). In all logistic regression models, a value of one indicated that a stomach contained at least one prey item or the presence of a prey category within individual Walleye diets. We ran three separate logistic regression models when evaluating the relationship between Walleye total length (2016 wild Walleye, 2016 stocked Walleye, and 2017 stocked Walleye) and the occurrence of stomachs containing at least one prey item. Additionally, we ran another nine logistic regression models that assessed whether total length of different Walleye cohorts (wild Walleye in 2016, stocked Walleye in 2016, and stocked Walleye in 2017) was related to the presence of benthic invertebrates, fish, or zooplankton in their diets. All statistical analyses were performed in R 3.2.2 (Integrated development for R. R-Studio, Inc., Boston, USA) at a significance level of $\alpha = 0.05$.

Results

Across sampling years, 590 age-0 Walleye were collected and underwent gastric lavage (2016: 442 Walleye; 2017: 148 Walleye) with wild Walleye making up 9.7% (n = 57) and stocked Walleye making up 90.3% (n = 533) of samples (Figure 6.1). In 2016, 54 wild Walleye were collected from East Okoboji while in 2017, we only collected two wild Walleye (East Okoboji = 1; West Okoboji = 1; Figure 6.1). Proportions of empty stomachs differed between wild and stocked Walleye during 2016 (2016: $F_{1,19} = 14.19$; $P = 0.001$) with stocked Walleye having higher mean (± 95% CI) proportion by number of empty stomachs (wild = 0.15 ± 0.09; stocked = 0.40 ± 0.10). However, days post-stocking (2016: $F_{1,19} = 0.04$; $P = 0.84$; 2017: $F_{1,17} =$

0.02; $P = 0.88$) and an interaction between days post-stocking and cohort (2016: $F_{1,19} = 3.41$; $P = 0.08$) did not influence the proportion of Walleye with empty stomachs.

Walleye consumed a variety of zooplankton, benthic invertebrates, and fishes (Table 6.1). In 2016, stocked Walleye consumed a wider variety of prey items relative to stocked Walleye in 2017 (Table 6.1). Various species of Cladoceran made up most of the zooplankton recovered from both wild and stocked Walleye diets (Table 6.1). A majority of benthic invertebrates recovered from Walleye diets included Amphipoda, Chironomidae, Ephemeroptera, and Odonata (Figure 6.2). Wild Walleye had higher average proportions of fish in their diets (wild: 0.48 ; stocked: 0.25) while stocked Walleye had higher proprotions of benthic invertebrates (wild: 0.12; stocked: 0.20; Figure 6.2). Calanoid, Cladoceran, and Rotifers made up small proportions of wild and stocked Walleye diets (Figure 6.2). Proportion of zooplankton in diets of wild and stocked Walleye both decreased over time (Figure 6.3 A, B). In 2016, the average proportions of zooplankton decreased across days post-stocking (2016: $F_{1,19} = 6.51$; $P = 0.019$) from 0.37 on day of stocking to 0.00 on day 49 (Figure 6.3 A). However, proportion of zooplankton in wild and stocked Walleye diets were similar (2016: $F_{1,19} = 0.003$; $P = 0.95$) and there was not a significant interaction between Walleye cohort and days post-stocking (2016: $F_{1,19} = 0.43$; $P = 0.52$; Figure 6.3 A). Conversely, in 2017, the proportion of zooplankton in stocked Walleye diets was consistently low on all sampling dates and not dependent on days post-stocking ($F_{1,17} = 0.96$; $P = 0.34$; Figure 6.3 B). In 2016, the proportion of benthic invertebrates was dependent on an interaction between days post-stocking and Walleye cohort ($F_{1,19} = 5.10$; $P = 0.04$; Figure 6.3 C); wild Walleye had consistent proportions of benthic invertebrates among days post-stocking (mean = 0.22; SD = 0.33) whereas the proportion of benthic invertebrates in stocked Walleye diets increased (Day 0: mean = 0.13; SD = 0.34; Day 49: mean = 0.56; SD = 0.51). In 2017,

proportion of benthic invertebrates in stocked Walleye diets (mean = 0.31) was higher compared to 2016 (mean = 0.81) but not related to days since stocking ($F_{1,17}$ = 0.16; P = 0.69; Figure 6.3 D). In 2016, wild Walleye had 1.9 times higher average proportions of fish in their diets (0.57) compared to stocked Walleye (0.30; $F_{1,19}$ = 9.28; P = 0.007; Figure 6.3 E). However, average proportion of fish in Walleye diets did not change with days post-stocking (2016: $F_{1,19}$ = 2.89; P = 0.11; 2017: $F_{1,17}$ = 0.001; P = 0.97) or an interaction of Walleye cohort and days post-stocking (2016: $F_{1,19}$ = 0.26; P = 0.61; Figure 6.3 E, F).

Two days after stocking, percent similarity values were high (62.9%) but decreased to 23.6% and 26.1 % on days 4 and 13, respectively (Figure 6.4). By days 38 and 40, percent similarity index values increased to 66.7% and 79.7%, respectively (Figure 6.4). On day 41, percent similarity index values decreased to 50.0%, followed by an increase on day 47 (67.9%), and a decrease on day 49 (40.6%; Figure 6.4).

Wild Walleye had total lengths ranging from 129-215 mm, whereas stocked Walleye had total lengths ranging from 94-228 mm (Figure 6.5). Walleye total length was also not related to whether a wild (2016: β = -0.03, Z = -1.29, P = 0.20) or stocked (2016: β = -0.001, Z = -1.28, P = 0.20; 2017: β = 0.0003, Z = 0.029, P = 0.98) Walleye had diets containing at least one prey item. Additionally, the presence of zooplankton (2016 wild: β = 0.026, Z = 1.04; P = 0.30; 2016 stocked: β = 0.003, Z = 0.26; P = 0.80; 2017 stocked: β = -0.049, Z = -1.57; P = 0.12), benthic invertebrates (2016 wild: β = 0.005, Z = 0.27; P = 0.78; 2016 stocked: β = 0.002, Z = 0.19; P = 0.85; 2017 stocked: β = -0.002, Z = -0.09; P = 0.93), or fish (2016 wild: β = -0.031, Z = -1.32; P = 0.19; 2016 stocked: β = -0.008, Z = -0.88; P = 0.38; 2017 stocked: β = 0.009, Z = 0.54; P = 0.59) was not related to Walleye total length.

Discussion

Relative to wild or hatchery fish reared on live forage, pellet-reared hatchery fish often consume less prey (Bachman, 1984; Porak, Johnson, Crawford, Renfro, Schoeb, Stout, Krause, & DeMauro, 2002; Pouder, Trippel, & Dotson, 2010; Reiriz, Nicieza, & Brañta, 1998; Sosiak, Randall, & McKenzie, 1979), have lower prey capture efficiencies (Diana, Diffin, Einfalt, & Wahl, 2018), consume fewer prey types (Diana et al., 2018; Sosiak et al., 1979), and are slower to switch to novel prey (Ersbak & Haase, 1983). The success of Walleye stocking programs is indirectly dependent on Walleye recognizing and successfully foraging on high caloric prey items that promote growth and improve condition and over winter energy reserves. Our results suggest that there is an acclimation period where stocked Walleye must adjust to novel environments and learn to forage on live, mobile prey, as indicated by initial high proportions of empty stomachs and low diet overlap with wild Walleye. Similarly, in a laboratory setting, pellet-reared Walleye took 5 d to transition to live prey (Wahl, Einfalt, & Hooe, 1995). Fish often cease feeding when introduced to a novel environment, and it can take a number of days before feeding begins again (Paszkowski & Olla, 1985). For instance, Pouder, Trippel, & Dotson (2010) reported high rates of empty stomachs in pellet-reared Largemouth Bass *Micropterus salmoides* within 7 d after stocking, but bass diets became similar to wild conspecifics after 14 days. Additionally, hatchery propagated Coho Salmon and Atlantic Salmon adapt from feeding on familiar artificial to unfamiliar natural prey within 5 days, (Paszkowski & Olla, 1985; Stradmeyer & Thorpe, 1987; Wahl, Einfalt, & Hooe, 1995). Therefore, the initial high proportions of empty stomachs in stocked Walleye is likely due to acclimation to a novel environment and lack of experience with unfamiliar prey.

The success of stocking initiatives is dependent on stocked individuals consuming dietary items that promote growth, as well as improved condition. In addition to having more empty

stomachs, stocked Walleye had higher proportions of lower caloric prey items (benthic invertebrates) and smaller proportions of fish in their diets relative to wild Walleye. Furthermore, over time, proportions of fish in stocked Walleye diets never reached proportions similar to those observed in wild Walleye diets. Thus, stocked Walleye diet composition may negatively influence post-stocking growth and condition. Similarly, Pouder, Trippel & Doddson (2010) and Porak et al. (2002) reported that wild fingerling Largemouth Bass had more fish in their diets relative to similarly-sized stocked hatchery-reared individuals. Switching from immobile prey items (i.e., zooplankton and benthic invertebrates) low in individual caloric value to mobile, prey items (i.e., fish) that individually have higher caloric value can result in reduced capture efficiency (Einfalt & Wahl, 1997; Galarowicz, Adams, & Wahl, 2006). Thus, there is a trade-off between consuming large numbers of easily captured prey low in individual nutritional value (e.g., zooplankton) or consuming prey with higher individual nutritional value (e.g., fish) that are more difficult to capture. Therefore, stocked Walleye may have higher proportions of benthic invertebrates and lower proportions of fish compared to wild Walleye due to the differences in capture efficiency (Wahl, Einfalt, & Hooe, 1995).

A reoccurring observation in post-stocking evaluations is initial dietary differences between hatchery and wild conspecifics that progressively increase in similarity over time. Generally, piscivores feeding on novel prey have increased capture success as their experience with that prey increases (Godin, 1978; Reiriz, Nicieza, & Brañta, 1998). Thus, over time, with increasing exposure to natural forage, stocked Walleye should have diets that progressively became more similar to wild conspecifics. In the present study, stocked and wild Walleye diets gradually become more similar as more time passed post-stocking. Similarly, hatchery and wild Largemouth Bass have diets that differ during the first seven days post-stocking (PSI = 0.48);

however, by 14 days post-stocking, the diets of the two cohorts became progressively similar (Pouder, Trippel & Doddson, 2010).

Walleye undergo two ontogenetic diet shifts, switching from zooplanktivory to benthivory and then piscivory (Galarowicz, Adams, & Wahl, 2006; Maloney & Johnson, 1957; Priegel, 1969). Diet composition for Walleye is partially dependent on body size as well as availability of appropriately sized prey (Hoxmeier, Wahl, Hooe, & Pierce, 2004; Mathias & Li, 1982; Priegel, 1969). However, our results indicate that fingerling (94-214 mm) and wild Walleye (129-215 mm) total length was not related to the presence of various prey items in their diets. Similarly, others have noted that as Walleye increase in size, they do not necessarily remain strictly piscivorous, with larger Walleye (50-150 mm) having diets that commonly include zooplankton and benthic invertebrates (Galarowicz & Wahl, 2005; Kolar, Wahl, & Hooe, 2003).

Inefficient foraging behavior has direct and indirect effects on the survival of recently stocked fishes, including reduced growth and prolonged duration in which young-of-year fishes are vulnerable to size-selective predation. Walleye at Iowa hatcheries have been observed to immediately consume live fish in the raceways (C. Clouse, Iowa DNR, *personal observation*). However, these observations may be Walleye responding to prey hitting the water surface, similar to the pellets they are accustomed to consuming, in a simple environment devoid of predators. Our results suggest that the proportion of fish in stocked Walleye diets was not similar to those of wild Walleye, suggesting that relative to wild conspecifics, stocked Walleye may have lacked foraging behaviors sufficient to capturing fish. Previous studies have suggested that feeding hatchery fish live forage can improve capture success, diet composition, color, and size at stocking (Colgan, Brown, & Orsatti, 1986; Ellis, Hughes, & Howell, 2002; Godin, 1978;

Ibrahim & Huntingford, 1992; Larscheid et al., 1999; Szendrey & Wahl, 1995). Furthermore, supplementing diets with live forage before stocking can reduce feeding behaviors, such as staying high in the water column for long durations, which can result in increased chances of post-stocking predation (Suboski & Templeton, 1989). After 21 days of starvation, stocked Walleye (45-230 mm) exhibit decreases in capture efficiency and increases in prey handling time as well as increases in vulnerability to predation (Jonas & Wahl, 1998). Transitioning Walleye to live prey in hatcheries for a short amount of time before stocking may improve their ability to capture live prey after stocking. While likely beneficial, providing live feed (i.e., minnows) to all hatchery Walleye is expensive and requires additional space and resources. Further evaluations assessing the link between foraging and exploratory behaviors of Walleye would provide useful information that could enhance our understanding as to whether altered diet composition could partially explain highly variable stocking success.

Recently, there has been a surge in studies evaluating whether "life skills" training during the rearing process can improve stocking success by promoting natural feeding behaviors on live forage (Sloychuk, Chivers, & Ferrari, 2016; Sundstom & Johnsson, 2001; Wisenden, Klitzke, Nelson, Friedl, & Jacobson, 2004). Evaluating the influence of propagation techniques (pellet trained versus live forage) on post-stocking diet composition is crucial for improving the survival and stocking success of hatchery-reared fishes. Pellet trained hatchery propagated Turbot *Scophthalmus maximus* (Ellis, Hughes, & Howell, 2002), Atlantic Salmon (Reiriz, Nicieza, Brañta, 1998), Largemouth Bass (Rachels et al., 2012), and Japanese Flounder *Paralichthys olivaceus* (Fujii & Noguchi, 1993) have been observed to have diets that differ from wild conspecifics, whereas hatchery-reared Largemouth Bass raised on live forage (i.e., minnows) before stocking have diets similar to wild conspecifics (Rachels et al., 2012). A challenge in

evaluating fall diets of wild and stocked Walleye is variable recruitment of wild fish as well as prey availability that can vary across systems as well as annually. Wild Walleye have variable mortality rates that can range from 84% to over 99% (McWilliams & Larscheid, 1992; Noble, 1972), which limited our ability to collect wild Walleye and directly compare dietary contents during both years. However, our results suggest that pellet reared stocked Walleye have higher occurrences of empty stomachs that declines with days since stocking. Lack of experience with live forage may result in initially high proportions of empty stomachs and diets containing prey items with low individual nutritional value. Thus, hatcheries propagating Walleye may consider supplementing walleye diets with live forage prior to stocking. Rearing practices that promote post-stocking feeding behaviors as well as improve fall growth and condition have the potential to improve fall post-stocking survival.

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Tables

Table 6.1 Mean proportion by number (± 95% confidence intervals) of fall prey items consumed by wild and stocked Walleye. Prey items were groups into the four prey categories (zooplankton, benthic invertebrates, fish, and other) for statistical analysis. Wild Walleye diets in 2017 were not included in the table due to the limited sample size (n = 1). Sample sizes (n) indicate the number of individual Walleye per cohort that were collected and had diet items recovered from their stomachs.

Prey Items	2016		2017	
	Wild (n = 44)	**Fingerling (n = 232)**	**Wild (n = 1)**	**Fingerling (n = 72)**
Zooplankton				
Calanoid	0.000 (0.000)	0.001 (0.002)	0.000 (0.000)	0.007 (0.014)
Cladoceran	0.059 (0.042)	0.077 (0.021)	0.000 (0.000)	0.009 (0.014)
Cyclopoid	0.013 (0.017)	0.001 (0.002)	0.000 (0.000)	0.000 (0.000)
Rotifer	0.001 (0.003)	0.015 (0.011)	0.000 (0.000)	0.003 (0.005)
Benthic Invertebrates				
Amphipoda	0.032 (0.027)	0.086 (0.022)	0.000 (0.000)	0.194 (0.053)
Chironomidae	0.024 (0.029)	0.009 (0.007)	0.000 (0.000)	0.024 (0.022)
Coleoptera	0.000 (0.000)	0.003 (0.005)	0.000 (0.000)	0.000 (0.000)
Diptera	0.000 (0.000)	0.007 (0.007)	0.000 (0.000)	0.007 (0.014)
Ephemeroptera	0.006 (0.008)	0.001 (0.001)	0.000 (0.000)	0.000 (0.000)
Hemiptera	0.000 (0.000)	0.008 (0.007)	0.000 (0.000)	0.000 (0.000)
Odonata	0.004 (0.007)	0.007 (0.007)	0.000 (0.000)	0.007 (0.014)
Trichoptera	0.000 (0.000)	0.012 (0.009)	0.000 (0.000)	0.000 (0.000)
Trombidiformes	0.000 (0.000)	0.003 (0.003)	0.000 (0.000)	0.000 (0.000)
Fish				
Centrarchidae	0.034 (0.038)	0.000 (0.000)	1.000 (0.000)	0.012 (0.016)
Cyprinidae	0.008 (0.015)	0.009 (0.008)	0.000 (0.000)	0.000 (0.000)
Sciaenidae	0.000 (0.000)	0.002 (0.004)	0.000 (0.000)	0.000 (0.000)
Other				
Hirudinea	0.000 (0.000)	0.003 (0.003)	0.000 (0.000)	0.018 (0.028)
Unidentifiable	0.051 (0.044)	0.093 (0.024)	0.000 (0.000)	0.045 (0.036)

Figures.

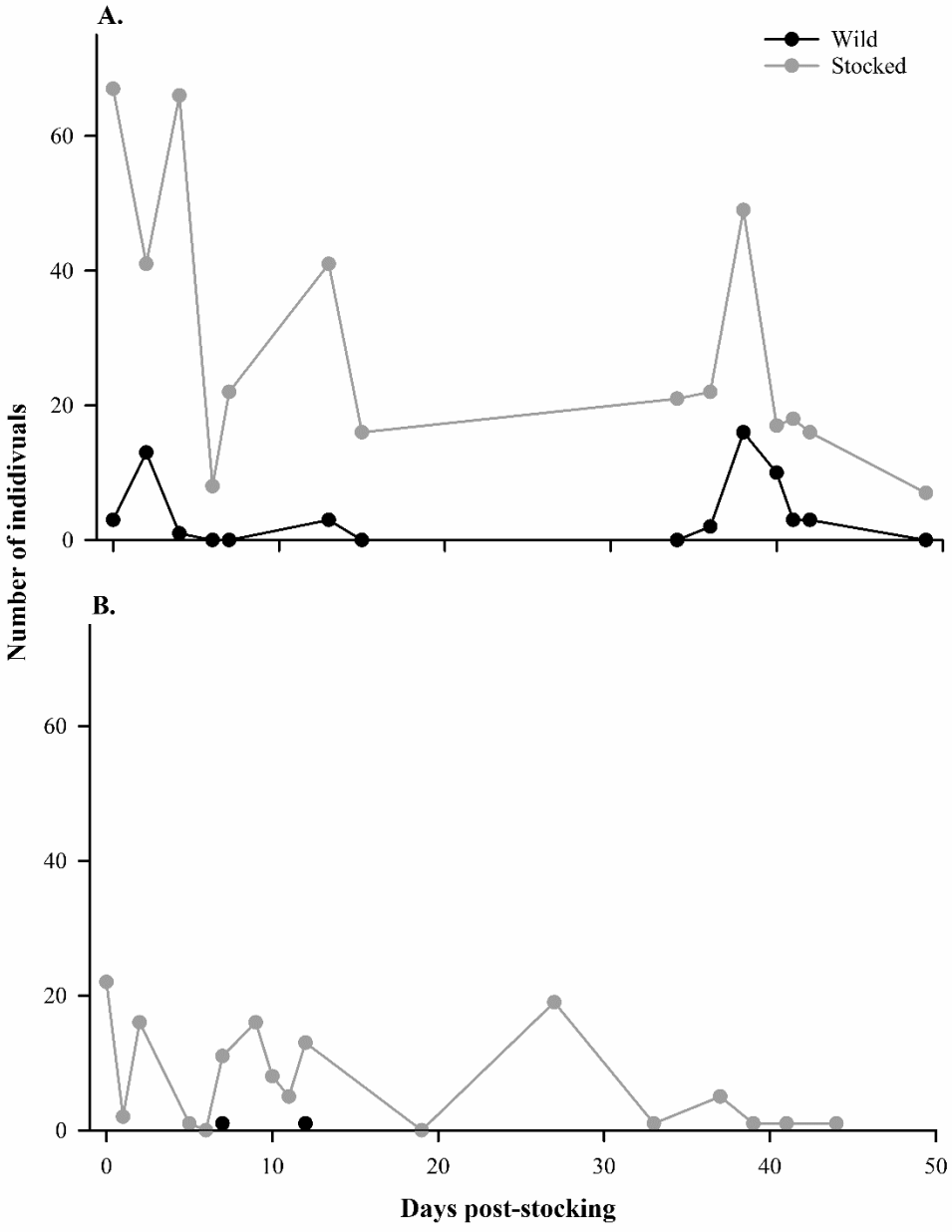

Figure 6.1 Number of wild (black circle with black solid line) and stocked Walleye (grey circle with grey solid line) collected with boat electrofishing in East Okoboji and West Okoboji in 2016 (A) and 2017 (B).

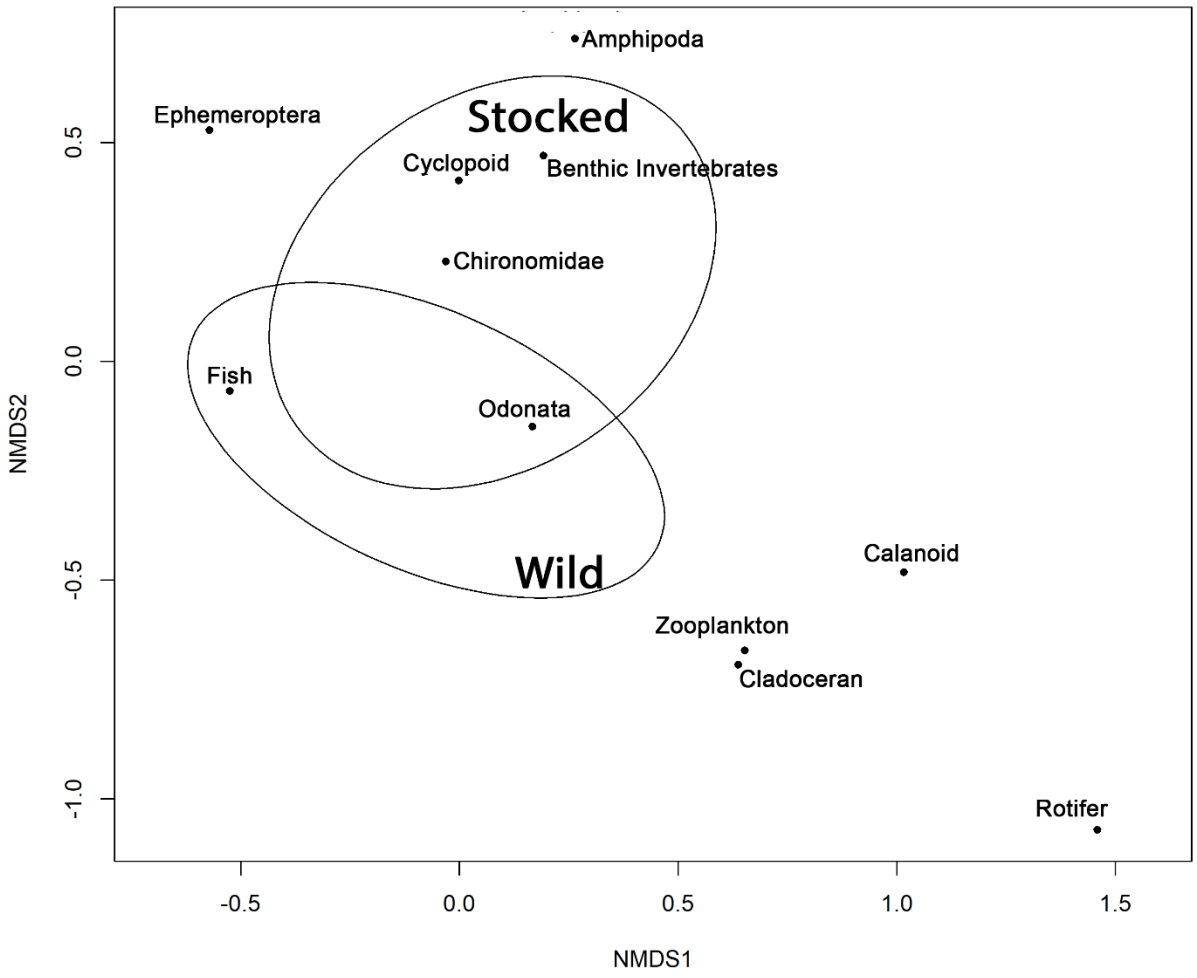

Figure 6.2 Non-Metric Multidimensional Scaling (NMDS) plot comparing proportions of common diet items (zooplankton, Calanoid, Cladoceran, Rotifer, Odonata, fish, Chironomidae, Cyprinidae, Cyclopoid, Invertebrate, Ephemeroptera, and Amphipoda) that made up wild and stocked Walleye. Broad classification of prey items (zooplankton, benthic invertebrates, and fish) account for proportions of individual identify prey groups as well as prey items that could only be classified broadly. All points specific to each Walleye cohort (wild versus stocked fingerling) are encircled with an ellipse.

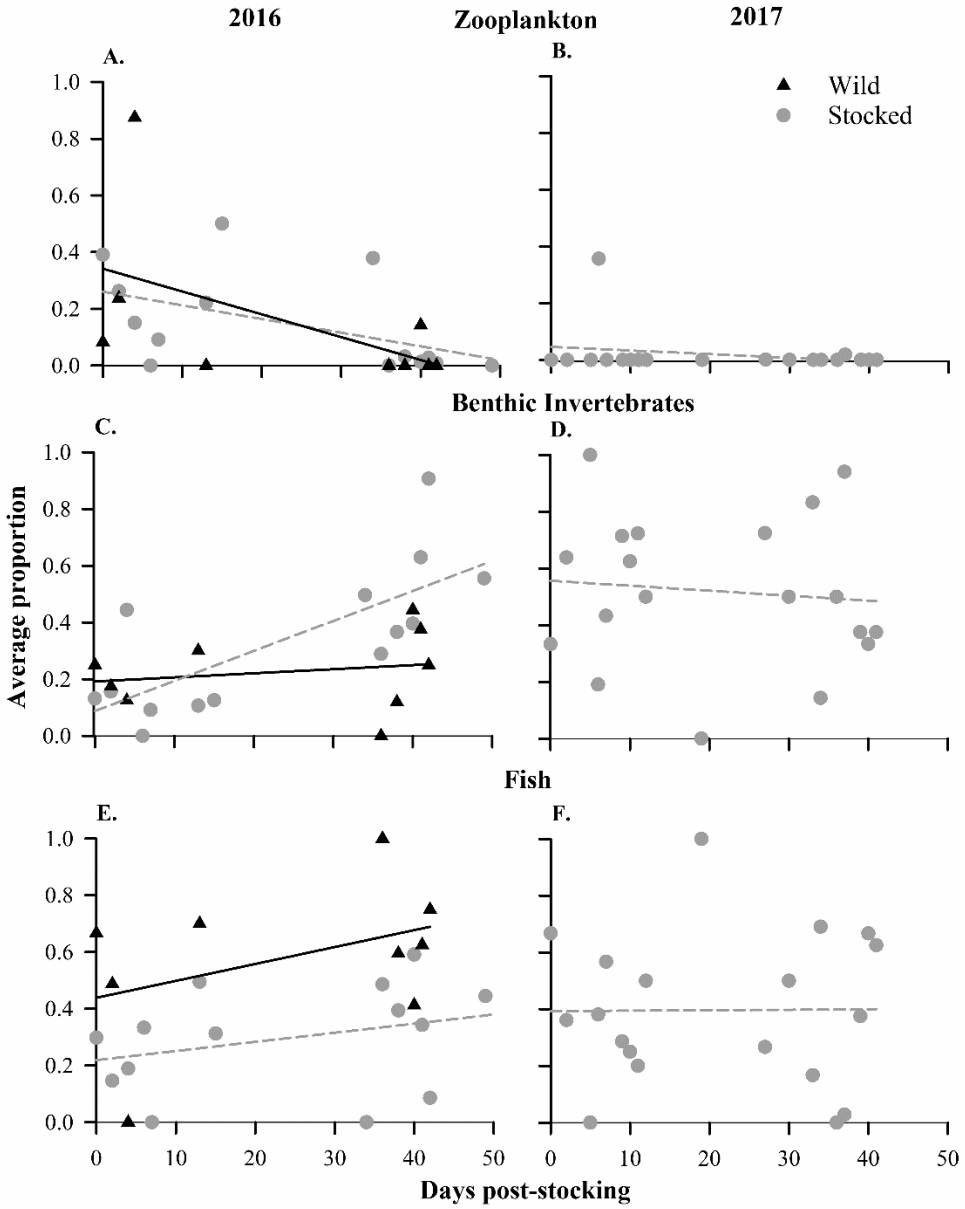

Figure 6.3 Proportions of zooplankton (top panels; A&B), benthic invertebrates (middle panels; C&D), and fish (bottom panels; E&F) in individual wild (black triangle) and fingerling (grey circles) Walleye following stocking events in 2016 (left panels) and 2017 (right panels). Regression lines represent temporal patterns in proportions of prey items for wild (solid black line) and stocked (dashed grey lines) Walleye. Only two wild Walleye were collected in 2017 and were not included in analyses or depicted in the figure.

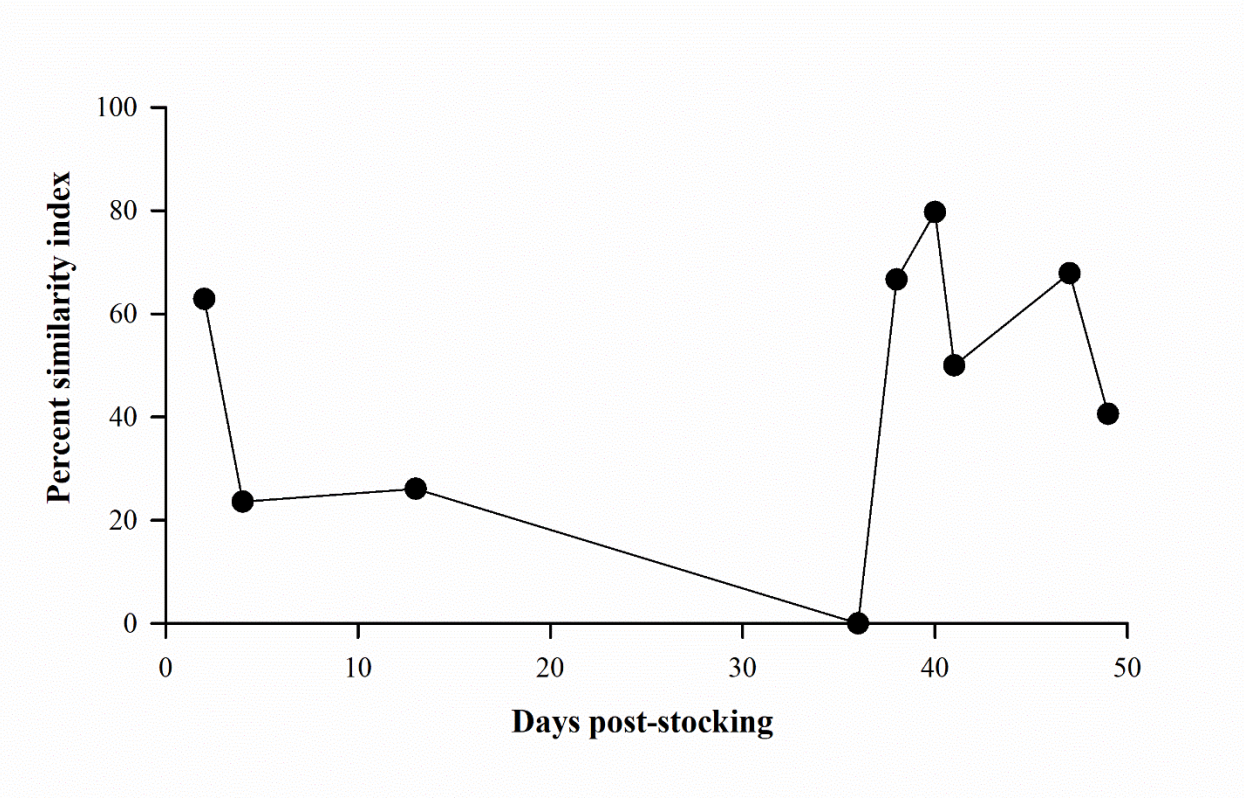

Figure 6.4 Percent similarity index values comparing diet composition of wild and stocked Walleye in 2016. The index values range from 0 (no similarity) to 100 (complete similarity).

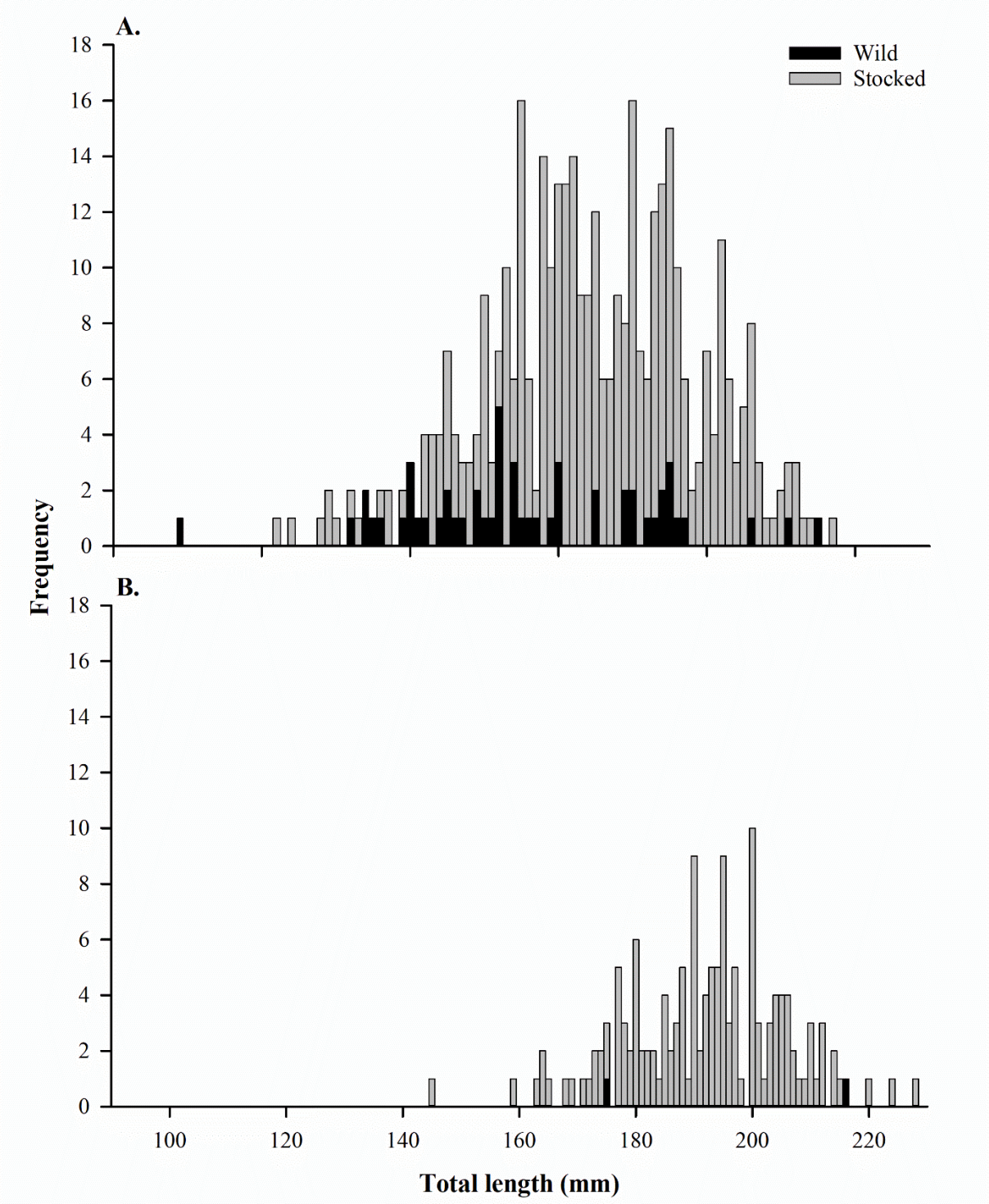

Figure 6.5 Length-frequency histograms of wild (black) and stocked (grey) Walleye collected with boat electrofishing in East Okoboji and West Okoboji during fall 2016 (A) and 2017 (B). In 2017, only two wild Walleye were captured.

CHAPTER 7. USING MARK-RECAPTURE TECHNIQUES TO ASSESS FALL WEEKLY SURVIVAL OF FALL STOCKED WALLEYE *SANDER VITREUS* IN TWO IOWA LAKES

Modified from a manuscript to be submitted to Fisheries Research

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Abstract

Stocking walleye *Sander virteus* is a common management technique to supplement populations where natural reproduction is limited. However, numerous factors can independently and dependently influence the survival of fall stocked walleye, including environmental conditions, predation, and stocked walleye total length, condition, and foraging ability. However, the relative effects of these factors on survival of stocked walleye are challenging to evaluate and rarely assessed simultaneously. Our objective was to evaluate apparent weekly survival of stocked fingerling walleye (98-287 mm TL) from stocking until ice cover in two Iowa systems (East Okoboji and West Okoboji) during fall 2015, 2016, and 2017 using mark-recapture techniques. Each fall, roughly 4,000 stocked walleye were implanted with passive integrated transponder tags, stocked, and recaptured via boat electrofishing. Cormack-Jolly-Seber recapture models estimated that walleye weekly apparent survival was negatively related to the weekly average proportion of walleye recovered from predator diets (β = -2.86; 95% credibility interval = -5.69 to -0.17) and weekly average water temperatures (β = 0.09; 95% credibility interval = -0.08 to 0.20). At an average fall water temperature, mean walleye apparent survival was 0.83 (95% credibility interval = 0.75 to 0.91) when no walleye were recovered from predator diets and declined to 0.54 (95% credibility interval = 0.28 to 0.84) when walleye made up 60% of

predator diets. Between 41-97% of stocked walleye were lost to mortality from stocking through ice-up, indicating that the two months post-stocking is a critical period for survival. Our results suggest that post-stocking walleye survival is most influenced by post-stocking predation rather than individual walleye or environmental factors. Thus, strategies that minimize predation following stocking may result in the greatest improvements in walleye survival.

Introduction

Stocking fish has been a management tool used since the mid-1800s (Hubert and Quist, 2010) to introduce, rehabilitate, supplement, or maintain fisheries (Kerr, 2011). However, a pitfall of stocking programs is variable post-stocking survival (Coghlan et al., 2007; Steffensen et al., 2010; Zelasko et al., 2010; Weber et al., 2020). Understanding underlying mechanisms that influence post-stocking survival provides insight as to how fisheries managers might circumvent variable post-stocking survival rates. Ultimately, small changes in post-stocking survival can result in substantial differences in year-class strength and recruitment, which can result in improved stocking success.

Numerous biotic and abiotic factors can influence survival during early life stages of fish. Factors that influence survival include predation (Santucci and Wahl, 1993; Buckmeier et al., 2005; Thompson et al., 2016), available forage (Johnson et al., 1996; Hoxmeier et al., 2006), competition (Le Pape and Bonhommeau, 2015; Chase et al., 2016), fish origin (Kellison et al., 2000; Jonsson and Jonsson, 2003; Pollock et al., 2007), body size (Litvak and Leggett, 1992; Meekan et al., 2006; Grausgruber and Weber, *in press*), and water temperatures (Akimova et al., 2016; Wagner et al., 2017). Furthermore, the aforementioned factors do not act independently of each other, making it challenging to determine their importance.

Walleye *Sander vitreus* is a highly sought-after sport fish with variable recruitment rates (Foust and Haynes, 2007; Hansen et al., 2015, 2017), making it challenging to maintain

productive fisheries. Thus, fisheries managers often supplement walleye populations with fingerling stockings (Kerr, 2011; Hoxmeier et al., 2006; Kampa and Hatzenbelor, 2009). However, fingerling walleye can have highly variable stocking success (Fielder, 1992; Jennings and Philipp, 1992; Mitzner, 1992; Kampa and Hatzenbelor, 2009). Environmental characteristics (lake water temperature at time of stocking and overall lake water temperature; Hoxmeier et al., 2006), predation (number and type of predators and consumption of alternative prey items; Freedman et al., 2012; Grausgruber and Weber, *in preparation*), and stocked walleye total length and condition (Santucci and Wahl, 1993; Mitzner, 2002; Uphoff et al., 2019; Grausgruber and Weber, *in press*) have been reported to influence walleye survival. Yet, the relative effects of all of these different factors compared to one another is unclear. Identifying factors that negatively influence post-stocking survival ultimately can result in improvements to rearing and stocking practices, which could result in improved stocking success and more sustainable fisheries.

Our objective included using mark-recapture modeling to estimate post-stocking apparent weekly survival rates of fingerling walleye stocked into two systems (East Okoboji and West Okoboji) during fall 2015, 2016, and 2017. We hypothesized that stocking environment (water temperature at time of stocking, weekly average water temperatures), predation (proportions of walleye in predator diets, total predator abundance, largemouth bass *Micropterus salmoides* abundance, northern pike *Esox lucius* abundance, and variety of prey types in predator diets), and characteristics of stocked walleye (total length and condition at time of stocking, and proportion of walleye with empty diets), would influence post-stocking survival. Unlike prior research evaluating the effects of individual parameters on fingerling walleye survival, our modeling approach provided a methodology for assessing the relative importance of individual parameters as well as their additive effects on post-stocking survival.

Methods

Study systems

East Okoboji and West Okoboji lakes are located in Dickinson County, Iowa. East Okoboji is a long, narrow, and shallow eutrophic natural lake (743 ha) with a 4,767-ha watershed that consists primarily of agriculture. The lakes basin slopes gradually and has a mean depth of 3.2 m and a maximum depth of 6.7 m. West Okoboji is a large oligotrophic natural lake (1,557 ha) with a 6,133-ha watershed that also consists primarily of agriculture. Relative to East Okoboji, West Okoboji has a more complex basin with a mean depth of 11.6 m and a maximum depth of 40.8 m. Submersed aquatic vegetation abundant in both East Okoboji and West Okoboji includes wild celery *Vallisneria americana,* flatstem pondweed *Potamogeton zosteriformis*, curly-leaf pondweed *Potamogeton crispus*, bushy pondweed *Najas flexillis*, clasping leaf pondweed *Potamogeton richardsonii*, and coontail *Ceratophyllum demersum*. Curly-leaf pondweed dominates the upper two basins of East Okoboji from late fall to early summer. Piscivore assemblages in East Okoboji and West Okoboji include largemouth bass, muskellunge *Esox masquinongy*, adult walleye, northern pike, and smallmouth bass *Micropterus dolomieu*. East Okoboji and West Okoboji are interconnected via a canal located on the southeast corner of West Okoboji.

Walleye stocking

East Okoboji and West Okoboji were stocked with fingerling walleye (98-287 mm; hereafter referred to as ‘walleye’) during fall 2015, 2016, and 2017. This project was conducted under the guidance and approval from Iowa State University Institutional Animal Care and Use Committee (Iowa State University IACUC 7-15-8051-I). Each year, approximately 30,000 walleye originating from a combination of two hatcheries (Spirit Lake Fish Hatchery and Rathbun Fish Hatchery) were stocked across both systems (2015: 31,440 walleye; 2016: 28,410

walleye; 2017: 30,461 walleye). Walleye from each hatchery were stocked at different times (Spirit Lake = mid-September; Rathbun = late October) and transported different durations (Spirit Lake = 2.6-18.2 km; Rathbun = 453.8-457.1 km). Each year before stocking, a subset of walleye from each hatchery (~2,000 individuals per year/ hatchery; 13-14%) were measured (total length; mm), weighed (g), and a 12-mm Hallprint food-grade passive integrated transponder (PIT) tag was inserted in the left dorsal musculature (Table 7.1). PIT tagging has little effect on walleye growth or survival (Weber and Flammang 2017). Condition at time of stocking (*K;* g/mm^3) was determined for all PIT-tagged individuals as:

$$K = \left(\frac{W}{TL^3}\right) * 10^5$$

where wet weight (g) is denoted as *W*, and total length (mm) is denoted as *TL* (Anderson and Neumann 1996).

Walleye were allowed to recover in hatchery raceways for 2-5 days following tagging. Walleye from the Spirit Lake Hatchery (2.6-18.2 km from stocking locations; hereafter referred to as 'Spirit Lake walleye') were stocked in late September (2015: September 24th; 2016: September 27th; 2017: September 28th) while walleye from the Rathbun Hatchery (453.8-457.1 km from systems; hereafter referred to as 'Rathbun walleye') were stocked in late October (2015: October 29; 2016: October 31; 2017: October 31). Walleye were not fed 48 hours before transportation to decrease waste (e.g., ammonia and carbon dioxide) during transportation (Robb 2008). The transportation truck had three 1,260 L compartments, all equipped with ram-air ventilation and supplemental oxygen (0.2 to 0.4 L min-1 with a maintained tank pressure at 276 kPa). Approximately 514 walleye (60.7 kg ± 0.25 kg) were transported in each tank during each stocking event, and PIT-tagged walleye were transported in conjunction with conspecifics that were not tagged. Rathbun Hatchery transportation truck tanks were equipped with OxyGuard

Pacific water temperature probe that recoded water temperature and dissolved oxygen every 18 minutes. Spirit Lake Hatchery transportation trucks were filled with East Okoboji Lake water immediately prior to transport and it was assumed water quality parameters underwent minor changes due to the short transport duration (2.6 to 18.2 km). Each year, daily average midwater temperatures for each system were collected hourly by centrally located HOBO Pendant logger.

Post-stocking monitoring

After stocking, largemouth bass, muskellunge, northern pike, smallmouth bass, adult walleye, and stocked fingerling walleye were captured via nightly boat electrofishing (220V, 5-8 amps pulsed DC) in each system until ice up (2015: September 24th to November 20th; 2016: September 27th to November 16th; 2017: September 28th to November 14th). Due to the large size of the systems (East Okoboji = 743-ha; West Okoboji = 1,557-ha) and limited post-stocking fingerling walleye movement patterns (mean dispersal from stocking location in East Okoboji was < 1,500 m by 35 days post stocking; Weber et al., 2020), our primary sampling region consisted of a 229-ha area in East Okoboji and a 152-ha area in West Okoboji; where physical features on lakes made it easy for field crews to identify primary sampling region. Additionally, periodic sampling outside our primary sampling region occurred to attempt to locate fish that moved out of the primary sampling area. However, catch rates of fingerling walleye outside our primary sample region were very low. Additionally, we did not catch any tagged walleye outside our primary sample region.

Upon capture, largemouth bass, muskellunge, northern pike, smallmouth bass, and adult walleye were measured (TL; mm), weighed (g), and stomach contents were non-lethally collected via pulsed gastric lavage (Grausgruber and Weber, *in press*, *in review*). Pulsed gastric lavage consisted of flushing each predator stomach until there were three sequential instances where nothing was recovered from the stomach. Regurgitated contents from predators were

concentrated on a 150-µm mesh screen, with prey items being preserved with 95% ethanol. In the lab, prey items were enumerated and identified to the lowest taxonomic level and classified broadly into the following prey categories: Ictaluridae, Centrarchidae, Decapoda, Cyprinidae, unidentifiable fish, Sciaenidae, adult Anura, Clupeidae, Insecta, larval Anura (tadpoles), unidentifiable, walleye, Moronidae, and Percidae. For each predator stomach sample, we enumerated the number of prey categories present (Grausgruber and Weber, *in review*). Additionally, for each predator, the proportion of walleye in predator diets was determined by dividing the wet weight of eaten walleye by the wet weight of all stomach contents recovered.

Predator catch-per-unit effort (CPUE) was calculated for each week across systems and years as:

$$CPUE = \frac{total\ number\ of\ indidivuals\ captured\ within\ a\ week}{total\ weekly\ shock\ time}$$

where the total number of individuals captured within a week was summarized as either the total number of all predators (largemouth bass, northern pike, muskellunge, smallmouth bass, and adult walleye), the total number of northern pike or largemouth bass captured within a week. Total weekly shock time (sec) was the summation of all nightly shock runs within a week.

Upon capture, stocked walleye were measured (TL; mm), weighed (g), scanned via an ISO RFID portable reader (Biomark Model 601, Biomark, Boise, Idaho), individual PIT tag numbers were recorded, and stomach contents were non-lethally collected via pulsed gastric lavage (Blankman et al., 2018). Tap water treated with Tetra AquaSafe (Tetra, Spectrum Brands) was used to displace stomach contents that were collected in Whirl-Pak bags and preserved with 95% ethanol. Each walleye underwent triplicate flushing of 140 mL/flush (total of 420 mL of water), and the contents from the triplicate flushing were pooled into one Whirl-Pak bag for each walleye (Blankman et al., 2018). Stomach contents from PGL and dissected stomachs were

concentrated separately by filtering them through a 63-µm-mesh Nitex filter, put into a plankton counting wheel, and identified under a dissecting microscope (Blankman et al., 2018).

Survival analysis

Apparent weekly survival (ϕ) and detection probability (p) of PIT-tagged walleye were estimated using daily live encounter histories in a Cormack-Jolly-Seber (CJS) open population model in Program MARK (White and Burnham, 1999) to generate maximum-likelihood estimates of weekly apparent survival (ϕ_j: conditional probability of surviving interval j provided the individual is alive and available for recapture during the interval; hereafter referred to as survival) and weekly detection probability (p_j: conditional probability of recapture in interval j given the individual is alive; White and Burnham, 1999). In this model, $1-\phi$ represents animals that died or emigrated from the population, as these two parameters cannot be estimated separately without additional information. The Cormack-Jolly-Seber model assumes tagged individuals are representative of the population to which inference is made, number of individuals tagged is known, tagging does not affect survival, releases and recaptures are made within brief time periods relative to the time between tagging, recapture does not affect subsequent survival or recapture, fates of individuals within and among cohorts are independent, and individuals in a group have the same survival and recapture probability for each time interval (White and Burnham, 1999).

Due to a large number of possible model structures for apparent survival and detection probability, running every possible model combination was impractical. Thus, we developed a set of a priori hypothesis to evaluate factors that may influence walleye survival and detection. Model-selection was based on Akaike's Information Criterion corrected for small sample size (AIC_c), where lower AIC_c values and higher Akaike weights (w_i) represent the most parsimonious model (Akaike, 1973). We characterized variation in weekly apparent survival and

weekly detection probability based on biological and abiotic factors hypothesized to influence young-of-year fish survival. Parameters hypothesized to influence walleye detection probability (*p*) included variation associated with systems (East Okoboji and West Okoboji; *sys*), years (2015, 2016, and 2017; *yr*), stocking event (*stocking*), sampling effort (total weekly boat electrofishing shock time within a system and year; *shock time*), average weekly water temperature within a system and year (℃; *water temp*), and a linear trend in time since stocking (*T*) representing increasing or decreasing survival in relation to days post-stocking. Parameters hypothesized to influence walleye apparent survival (ϕ) included factors related to predation (proportions of walleye in predator diets [*WAE in diet*], total predator [largemouth bass, muskellunge, northern pike, smallmouth bass, and adult walleye] relative abundance [*Pred CPUE*], largemouth bass relative abundance [*LMB CPUE*], northern pike relative abundance [*NOP CPUE*], and variety of prey types in predator diets [*prey type*]), characteristics specific to stocked walleye (total length [mm; *TL*] and condition [*K*] at time of stocking and proportion of walleye with empty diets [*proportion empty*]), and stocking environment (water temperature at time of stocking [℃; *stocking water temp*], average weekly water temperatures [℃; *water temp*]). All parameters related to predation and environment consisted of weekly averages specific to a given year and system, whereas parameters specific to stocked walleye (total length and condition at the time of stocking) were based on individuals PIT-tagged fish. To avoid multi-collinearity of factors within a model, candidate models could only consist of a single factor from our list of parameters (predation, characteristics of stocked walleye, and environment). Thus, a candidate model including predation could only consist of either weekly average proportion of walleye in predator diets [*WAE in diet*], total predator abundance [*Pred CPUE*],

largemouth bass abundance [*LMB CPUE*], northern pike abundance [*NOP CPUE*], or variety of prey types in predator diets [*prey type*] and not multiple combinations of these predation metrics.

During the initial modeling phase, we ran a set of candidate models where apparent survival model structure was held constant, where apparent survival was dependent on an interaction between cohorts, systems, years, and time. We then optimized detection probability in model form in Program MARK using the logit link function and compared models using Akaike's Information Criterion corrected for small sample size (AIC_c; Burnham and Anderson 1998). Akaike weights (w_i) were calculated to address potential uncertainty concerning the selection of the top model (models 21-33; Table 7.2). With detection optimized, we then held the detection probability structure constant and optimized apparent survival (models 1-21; Table 7.2; Doherty et al. 2012). Similar to our approach for optimizing detection probability, competing hypotheses were stated in model form using the logit link function and compared using Akaike's Information Criterion corrected for small sample size (AIC_c; Burnham and Anderson, 1998). Akaike weights (w_i) were calculated to address potential uncertainty concerning the selection of the top model (Burnham and Anderson, 1998). Markov Chain Monte Carlo (MCMC) simulations were run for the most supported model (model 1): original maximum likelihood parameter estimates from the top model were used as starting values, and the simulation ran 4,000 tuning iterations, followed by 1,000 burn-in iterations, and 10,000 iterations that were used in the final estimates. All results are reported as mean parameter values and 95% credibility intervals from the simulations. Cumulative survival estimates were based on a hypothetical 1,000 walleye and mean weekly apparent survival estimates and their credibility intervals.

Results

Stocked walleye

The number of walleye recaptured varied across years, systems, and cohorts (Table 7.1). Total number of recaptures was highest in 2016 (total = 100 walleye), followed by 2017 (total = 15 walleye), and 2015 (total = 14 walleye). In general, the percent recapture was low, with values ranging from 0.1-5.3% (Table 7.1). However, the average recapture from the first stocking event (Spirit Lake Hatchery) was 1.7 times greater than those of the second stocking event (Rathbun Hatchery; Table 7.1). Walleye in the first stocking event had total lengths ranging from 102-286 mm, whereas walleye in the second stocking event had total lengths ranging from 98-287 mm. Condition at the time of stocking was similar for both stocking events, with walleye in the first stocking event having condition values ranging from 0.5-6.6 g/mm^3 and walleye in the second stocking event had condition values ranging from 0.5-6.3 g/mm^3.

Survival

A total of 33 candidate models were evaluated to compare different biological and abiotic effects on post-stocking walleye weekly apparent survival and detection probability (Table 7.2). Only two candidate models had $\Delta AIC_c < 2.0$ and $w_i > 0.30$, indicating support for those models (Table 7.2). Models ranked 3 to 33 had ΔAIC_c values (≥ 2.00) and smaller w_i values (≤ 0.07), indicating little support (Table 7.2). The top model indicated that walleye detection probability was positively related to total amount of weekly boat electrofishing shock time ($\beta = 0.04$; 95% credibility interval = 0.03 to 0.05). Stocking event was in the most supported model for detection probability (Table 7.2) but the slope of the relationship did not differ from zero ($\beta = 0.00002$; 95% credibility interval = -0.0009 to 0.0009). Average weekly detection probabilities for walleye were low, with an average value of 0.005 (min. = 0.002; max. = 0.150; Figure 7.1). While optimizing detection probability, models consisting of various combinations of system, cohort,

year, trend (*T*), and average weekly water temperature (*water temp*) effects on detection probability were evaluated (models 22-33) but received no support ($\Delta AIC_c > 99$). Thus, these parameters were not included as factors on detection probability in models evaluating apparent weekly survival (Table 7.2).

The best supported model indicated that walleye apparent weekly survival was negatively related to the weekly average proportion of walleye recovered from predator diets ($\beta = -2.86$; 95% credibility interval = -5.69 to -0.17; Table 7.2). Additionally, walleye apparent survival and weekly average water temperature were positively related to weekly apparent survival (Table 7.2); however, the slope of the relationship did not differ from zero ($\beta = 0.09$; 95% credibility interval = -0.08 to 0.20). At an average fall water temperature, mean weekly walleye apparent survival was 0.83 (95% credibility interval = 0.75 to 0.91) when no walleye were recovered from predator diets (Figure 7.2). Apparent weekly survival declined to 0.54 (95% credibility interval = 0.28 to 0.84) when walleye made up 60% of predator diets; however, credibility intervals of apparent survival estimates overlapped when walleye made up 0 and 60% of predator diets (Figure 7.2). At the mean proportion of walleye in predator diets (mean = 0.11), mean walleye apparent survival increased with increasing water temperatures (Figure 7.2). At 4°C, mean walleye survival was 0.24 (95% credibility interval = 0.06 to 0.59) while at 18°C mean walleye survival was 0.95 (95% credibility interval = 0.74 to 0.99; Figure 7.2).

After the first stocking event in East Okoboji, apparent weekly survival of walleye generally remained static; however, weekly apparent survival declined at week 6 in 2015 and week 4 in 2017. In East Okoboji during 2015, weekly apparent survival rates went from 0.89 (95% credibility interval = 0.79-0.94) on week five to 0.69 (95% credibility interval = 0.51 to 0.88) during week six whereas in 2017, weekly apparent survival rates went from 0.82 (95%

credibility interval = 0.73 to 0.90) on week four to 0.72 (95% credibility interval = 0.54 to 0.92) during week five (Figure 7.3). Following the first stocking event in West Okoboji, weekly apparent survival increased two weeks post-stocking in 2016 and 2017; however, apparent weekly survival rates in 2015 were static following the first stocking event and then decreased following on week 6 (Figure 7.3). In West Okoboji during 2016, weekly apparent survival rates increased from 0.66 (95% credibility interval = 0.41 to 0.93) on week one to 0.87 (95% credibility interval = 0.69 to 0.94) during week two. In 2017, weekly apparent survival rates increased from 0.61 (95% credibility interval = 0.33 to 0.94) on week one to 0.85 (95% credibility interval = 0.76 to 0.93) during week two (Figure 7.3).

By week seven, cumulative survival rates for the first stocking event were relatively low. Specifically, by week seven, post-stocking cumulative survival rates for the first stocking event ranged from 0.08-0.55 in 2015, 0.12-0.56 in 2016, and 0.03-0.59 in 2017 in East Okoboji (Figure 7.4). In West Okoboji, post-stocking cumulative survival rates for the first stocking event by week seven ranged from 0.07-0.54 in 2015, 0.07-0.55 in 2016, and 0.02-0.56 in 2017 (Figure 7.4). For the second stocking event, in East Okoboji cumulative survival by week 7 ranged from 0.23 to 0.74 in 2015, 0.49 to 0.83 in 2016, and 0.21 to 0.91 in 2017 (Figure 7.4). In West Okoboji cumulative survival by week 7 ranged from 0.18 to 0.70 in 2015, 0.51 to 0.82 in 2016, and 0.33 to 0.88 in 2017 (Figure 7.4).

Discussion

High post-stocking survival rates of fishes are advantageous economically and assist fisheries managers in meeting their management goals. However, numerous biotic and abiotic factors can independently and dependently interact with one another, making it challenging to determine how each factor influences post-stocking survival. Our results indicate that fall stocked walleye apparent survival was dependent on the average weekly proportion of walleye

recovered from predator diets. Initially, we hypothesized that consumption of walleye would negatively influence survival. Our top candidate model indicated that there was a negative relationship between apparent weekly survival rates of walleye to the weekly average proportion of walleye recovered from largemouth bass, smallmouth bass, northern pike, adult walleye, and muskellunge diets. Largemouth bass (Santucci and Wahl, 1993; Freedman et al., 2012) and northern pike (Johnson et al., 1996) have been documented to consume large percentages (0-49%) of stocked walleye populations. Furthermore, largemouth bass, northern pike, and adult walleye in East Okoboji and West Okoboji have been documented to collectively consume 2.4-27.2% of stocked walleye (220-300 mm) within one month after stocking (Grausgruber and Weber, *in review*). Our results here indicate that this prolonged predation pressure is the primary factor that influenced survival of stocked walleye, whereas the effects of other environmental and individual walleye factors on walleye survival received little support.

Water temperatures during the first growing season have repeatedly been hypothesized to influence growth, condition, and survival of age-0 Walleye, especially those in late spring and summer (Busch et al., 1975; Serns, 1982; Kocovsky and Carline, 2001). Sudden changes in water temperature can elicit stress responses in fish, even when values are within the tolerance range of the species (Wedemeyer, 1997) and can negatively influence survival (Harmon 2009). For walleye, temperature increases of 2-12 ℃ can result in physiological changes indicative of stress as well as increased mortality (Clapp et al., 1997; Ball et al., 2020). We initially hypothesized that warmer water temperatures at the time of stocking, as well as throughout the fall, could negatively influence post-stocking survival of walleye. However, our top two models suggested that weekly average water temperatures were positively related to weekly apparent survival. Walleye are a cool water fish species with growth occurring between 22-26℃ and

lethal water temperatures at 29-35℃ (Huh et al., 1976; Hokanson, 1977; Hokanson and Koenst, 1986). In the current study, weekly average water temperatures ranged from 7-18℃. Thus, water temperatures were well within ranges appropriate for walleye which could partially explain why there was not a negative relationship between post-stocking survival and water temperatures.

We observed cumulative apparent survival rates for initial stocking events ranging from 0.03 to 0.59 while cumulative apparent survival rate from the second stocking event ranged from 0.18 to 0.88. Alternatively, fingerling walleye (> 200 mm) radio-tagged and stocked across multiple Iowa reservoirs had a mean apparent cumulative survival of 0.70 at 49 d post-stocking (Weber et al., 2020). The success of walleye stocking programs depends upon the health and survival of the individuals released. In the current study, mean weekly apparent survival varied across weeks with weekly apparent survival estimates being initially low following the first stocking events followed by secondary decreases associated with the second stocking events. However, between stocking events, weekly apparent survival rates did not change drastically. Similarly, Weber et al. (2020) reported that daily apparent survival rates were the lowest immediately following fingerling walleye (mean TL ± SE = 235.2 ± 2.3 mm) stocking events; however, overall weekly apparent survival rates were relatively high (mean: 0.98 to 1.00). Similarly, we observed fairly high weekly apparent survival rates (mean: 0.77 to 0.83). Results from both studies suggest that average weekly apparent survival rates of fall stocked fingerling Walleye are relatively high. However, decreased weekly survival rates following stocking events suggests that the first two weeks following stocking events may be a critical to stocking success due to increases in weekly apparent survival between weeks one and two following stocking events.

There has been extensive research evaluating walleye characteristics that influence survival, such as total length (Santucci and Wahl 1993; Weber et al., 2020; Grausgruber and Weber *in press*) and condition (Jonas and Wahl 1998; Thompson et al., 1991). Generally, larger fish have decreased vulnerable to predation and starvation (i.e., bigger-is-better hypothesis; Rice et al. 1987; Miller et al. 1988). We hypothesized that walleye total length and condition at the time of stocking both would be positively related to post-stocking survival. However, we found little support suggesting that total length and condition at the time of stocking influenced post-stocking apparent survival. Prior evaluations indicate that predation risk decreases with increasing walleye total length (TL < 220mm; Grausgruber and Weber, *in press*) and that predators collectively consume significant proportions smaller walleye (Grausgruber and Weber, *in review*), suggesting that post-stocking survival is partially dependent on walleye total length. Low body condition at the time of stocking can result in an increased likelihood of post-stocking starvation. Walleye (45-230 mm) that experience prolonged starvation (21 d) can exhibit decreased prey capture efficiency and increases in handling and capture times (Jonas and Wahl, 1998). Stocking walleye with high body condition could abate the rate of post-stocking starvation, thereby increasing the time for hatchery-reared walleye to learn natural feeding behavior. Relative to fall diets of wild Walleye, fall stocked fingerling Walleye have been observed to have higher proportions of empty stomachs and lower proportions of high caloric prey items, such as fish, in their diets (Grausgruber and Weber, *in preparation*). However, our models suggested that the proportion of Walleye with empty stomachs was not a driving factor that influences post-stocking apparent weekly survival.

A significant challenge inherent in all mark-recapture studies is the ability to recapture tagged individuals and ensuring that model assumptions are met. We used individual PIT-tagged

walleye encounter histories to estimate apparent survival, which includes both mortality and emigration, as these two parameters cannot be estimated independently in CJS models (White and Burnham, 1999). Therefore, if walleye permanently moved to deeper waters where we could not effectively sample or left the sampling regions where a majority of our sampling efforts took place, then survival estimates would have been biased low. Thus, apparent survival estimates reported here are likely conservative, and true survival was likely higher. Despite intensive sampling each year, we were only able to recapture a low percent of stocked fish (0.1-5.3%). However, stocked fingerling walleye generally do not disperse far post-stocking (Weber et al., 2020) and additional sampling in subsequent years post-stocking has resulted in very few recaptures (< 5 fish; E. Grausgruber, *unpublished data*), providing additional evidence that survival of these stocked fish was low. Nonetheless, low recaptures of stocked Walleye resulted in imprecise survival estimates, making it difficult to accurately assess the fates of these fish. Alternative tagging techniques with improved detection (e.g., radio telemetry; Weber et al. 2020; Weber and Weber, *in press*) can result in more precise estimates of detection probability and apparent survival (White and Burnham, 1999). However, these tagging techniques are often limited in sample size that can also result in imprecise estimates and limited inference. Thus, additional work evaluating the trade-offs between sample size and detection probability for estimating survival rates of stocked fishes would be beneficial.

Assessing post-stocking survival of hatchery fishes is improves our understanding and identification of potential sources of mortality that can result in recruitment success or failure. Walleye apparent survival was negatively related to predator consumption of walleye, suggesting that post-stocking predation is a primary factor that influences stocking success. Moreover, the lack of support for factors related to environment and characteristics of stocked walleye agrees

with other publications indicating that these factors are not overly influential in post-stocking walleye survival (Jennings and Phillip, 1992; Olson et al., 2000; Ball et al. 2020). However, prior evaluations are generally limited by their assessments of single parameters hypothesized to influence post-stocking walleye survival, such as only evaluating the effects of post-stocking predation (Grausgruber and Weber, *in press, in review*) or post-stocking diet composition (Grausgruber and Weber, *in preparation*). While this single parameter approach provides useful information, it is limited in its ability to inform fisheries managers on the overall importance of each parameter. Our mark-recapture modeling approach allowed us to overcome the limitations of studies evaluating individual parameters and allowed us to simultaneously and individually assess the significance of parameters hypothesized to limit post-stocking walleye survival. Overall, our results suggest that fall post-stocking predation plays a more critical role in post-stocking survival and stocking success of walleye relative to environmental variables and walleye characteristics. Thus, fisheries biologists, managing systems with erratic walleye recruitment should initially consider evaluating predator densities near current stocking locations as well as at alternative stocking locations in effort to determine stocking locations that may have decreased occurrences of post-stocking predation. Additionally, fisheries biologists could consider stocking walleye across multiple locations in attempts to avoid stocking all the walleye in locations with high predator densities.

Acknowledgments

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Tables

Table 7.1 Number of hatchery-reared fingerling walleye stocked into East Okoboji and West Okoboji during fall 2015, 2016, and 2017 as well as weekly, total, and total percent recapture rates. Walleye average (±SD) total length (mm; TL) and condition (g/mm^3; K) values for each stocking group are provided, as well as total boat electrofishing shock time (hrs) during a given year and system. Hatchery denotes the fish hatchery (Spirit Lake or Rathbun) walleye rearing occurred. Asterisks indicate the week in which stocking occurred for the second stocking event of Rathbun Hatchery walleye.

				Weekly number of recaptures											
Year	**Hatchery**	**East Okoboji**	**West Okoboji**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Total recaptures**	**Total % recapture**	**TL (mm)**	**Condition (g/mm^3)**	**Total shock time (hrs)**
2015															
	Spirit	1,000		0	0	0	1	0	5	0	6	0.6	191 (13)	0.86 (0.07)	13.6
			1,007	0	0	0	0	0	0	1	1	0.1	191 (13)	0.82 (0.06)	14.9
	Rathbun	1,017						*	2	0	2	0.2	238 (17)	0.85 (0.06)	10.2
			1,014					*	2	3	5	0.5	239 (17)	0.83 (0.05)	14.4
2016															
	Spirit	996		4	5	0	0	28	9	7	53	5.3	208 (36)	0.87 (0.06)	38.0
			995	6	1	0	0	3	1	1	12	1.2	209 (36)	0.86 (0.06)	24.1
	Rathbun	992						*	11	2	12	1.2	211 (36)	0.87 (0.06)	35.0
			988					*	21	2	23	2.3	213 (36)	0.87 (0.18)	14.1
2017															
	Spirit	1,000		6	0	1	0	2	0	0	9	0.9	181 (12)	0.89 (0.07)	39.3
			1,011	0	2	0	1	0	0	0	3	0.3	181 (12)	0.89 (0.27)	36.0
	Rathbun	1,000						*	1	0	1	0.1	231 (15)	0.90 (0.21)	11.0
			1,000					*	2	0	2	0.2	234 (14)	0.84 (0.06)	15.5

Table 7.2 Cormack-Jolly-Seber models used to estimate apparent daily survival (ϕ) and detection probability (*p*) of walleye stocked into East Okoboji and West Okoboji in Iowa, USA during fall 2015, 2016, and 2017. Parameters hypothesized to influence walleye detection probability (*p*) included variation associated with systems (East Okoboji and West Okoboji; *sys*), years (2015, 2016, and 2017; *yr*), stocking event (*stocking*), sampling effort (total weekly boat electrofishing shock time within a system and year; *shock time*), average weekly water temperature within a system and year (°C; *temp*), and a linear trend in time since stocking (*T*). Parameters hypothesized to influence walleye apparent survival (ϕ*)* included factors related to predation (proportions of walleye in predator diets [*WAE in diet*], total predator relative abundance [*Pred CPUE*], largemouth bass relative abundance [*LMB CPUE*], northern pike relative abundance [*NOP CPUE*], and variety of prey types in predator diets [*prey type*]), characteristics of the stocked walleye (proportion of walleye with empty diets [*proportion empty*]; total length [mm; *TL*] and condition [g/mm^3; *K*] at time of stocking), stocking environment (water temperature at time of stocking [°C; *stocking water temp]*, and fall water temperatures [°C; *water temp*]), and no effect of any parameters (.). Model comparisons were based on AIC_c, ΔAICc, AIC_c model weight (AIC_c w_i), model likelihood, number of parameters (K), and deviance = -2 · log-likelihood of the model less -2 · log-likelihood of the saturated model (same number of parameters and degrees of freedom). In total we evaluated 33 candidate models.

Model Number	Model	AIC_c	ΔAIC_c	AIC_c w_i	Model Likelihood	K	Deviance
1	ϕ (WAE in diet + water temp), *p* (shock time + stocking)	48,060.56	0.00	0.47	1.00	6	48,048.55
2	ϕ (WAE in diet + water temp + K), *p* (shock time + stocking)	48,061.03	0.47	0.37	0.79	7	48,047.02
3	ϕ (water temp), *p* (shock time + stocking)	48,064.25	3.69	0.07	0.16	5	48,054.24
4	ϕ (WAE in diet), *p* (shock time + stocking)	48,066.54	5.98	0.02	0.05	5	48,056.54
5	ϕ (WAE in diet + K), *p* (shock time + stocking)	48,066.61	6.05	0.02	0.05	6	48,054.60
6	ϕ (WAE in diet + proportion empty), *p* (shock time + stocking)	48,068.35	7.79	0.01	0.02	6	48,056.34
7	ϕ (WAE in diet + TL), *p* (shock time + stocking)	48,068.54	7.98	0.01	0.02	6	48,056.53
8	ϕ (WAE in diet + stocking water temp), *p* (shock time + stocking)	48,068.54	7.98	0.01	0.02	6	48,056.54
9	ϕ (K), *p* (shock time + stocking)	48,070.24	9.68	0.00	0.01	5	48,060.23
10	ϕ (prey type), *p* (shock time + stocking)	48,070.59	10.03	0.00	0.01	5	48,060.58
11	ϕ (T), *p* (shock time + stocking)	48,071.21	10.65	0.00	0.00	5	48,061.21
12	ϕ (stocking water temp), *p* (shock time + stocking)	48,071.35	10.79	0.00	0.00	5	48,061.35
13	ϕ (.), *p* (shock time + stocking)	48,071.94	11.38	0.00	0.00	4	48,063.94
14	ϕ (T + TL), *p* (shock time + stocking)	48,072.45	11.89	0.00	0.00	6	48,060.45
15	ϕ (TL), *p* (shock time + stocking)	48,073.87	13.31	0.00	0.00	5	48,063.86
16	ϕ (NOP CPUE), *p* (shock time + stocking)	48,073.93	13.37	0.00	0.00	5	48,063.92
17	ϕ (Pred CPUE), *p* (shock time + stocking)	48,074.03	13.47	0.00	0.00	5	48,064.03
18	ϕ (LMB CPUE + TL), *p* (shock time + stocking)	48,075.94	15.38	0.00	0.00	6	48,063.94
19	ϕ (Pred CPUE + TL), *p* (shock time + stocking)	48,075.97	15.41	0.00	0.00	6	48,063.96
20	ϕ (LMB CPUE), *p* (shock time + stocking)	48,076.22	15.66	0.00	0.00	5	48,066.21
21	ϕ (proportion empty), *p* (shock time + stocking)	48,076.27	15.71	0.00	0.00	5	48,066.27
22	ϕ (g*t), *p* (shock time + stocking)	48,153.27	92.71	0.00	0.00	87	47,978.00
23	ϕ (g*t), *p* (shock time + water temp)	48,159.80	99.24	0.00	0.00	87	47,984.53
24	ϕ (g*t), *p* (sys)	48,159.97	99.41	0.00	0.00	86	47,986.73
25	ϕ (g*t), *p* (water temp)	48,164.43	103.87	0.00	0.00	86	47,991.19
26	ϕ (g*t), *p* (stocking)	48,165.97	105.41	0.00	0.00	86	47,992.73
27	ϕ (g*t), *p* (year)	48,172.05	111.49	0.00	0.00	87	47,996.78
28	ϕ (g*t), *p* (yr + sys)	48,173.14	112.58	0.00	0.00	90	47,991.78

Table 7.2 (Continued)

Model Number	Model	AIC_c	ΔAIC_c	AIC_c w_i	Model Likelihood	K	Deviance
29	ϕ (g*t), *p* (shock time)	48,175.96	115.40	0.00	0.00	86	48,002.72
30	ϕ (g*t), *p* (stocking + sys)	48,179.81	119.25	0.00	0.00	88	48,002.51
31	ϕ (g*t), *p* (yr + stocking)	48,182.84	122.27	0.00	0.00	90	48,001.48
32	ϕ (g*t), *p* (T)	48,213.96	153.40	0.00	0.00	86	48,040.72
33	ϕ (g*t), *p* (stocking + water temp)	48,216.70	156.14	0.00	0.00	87	48,041.43

Figures

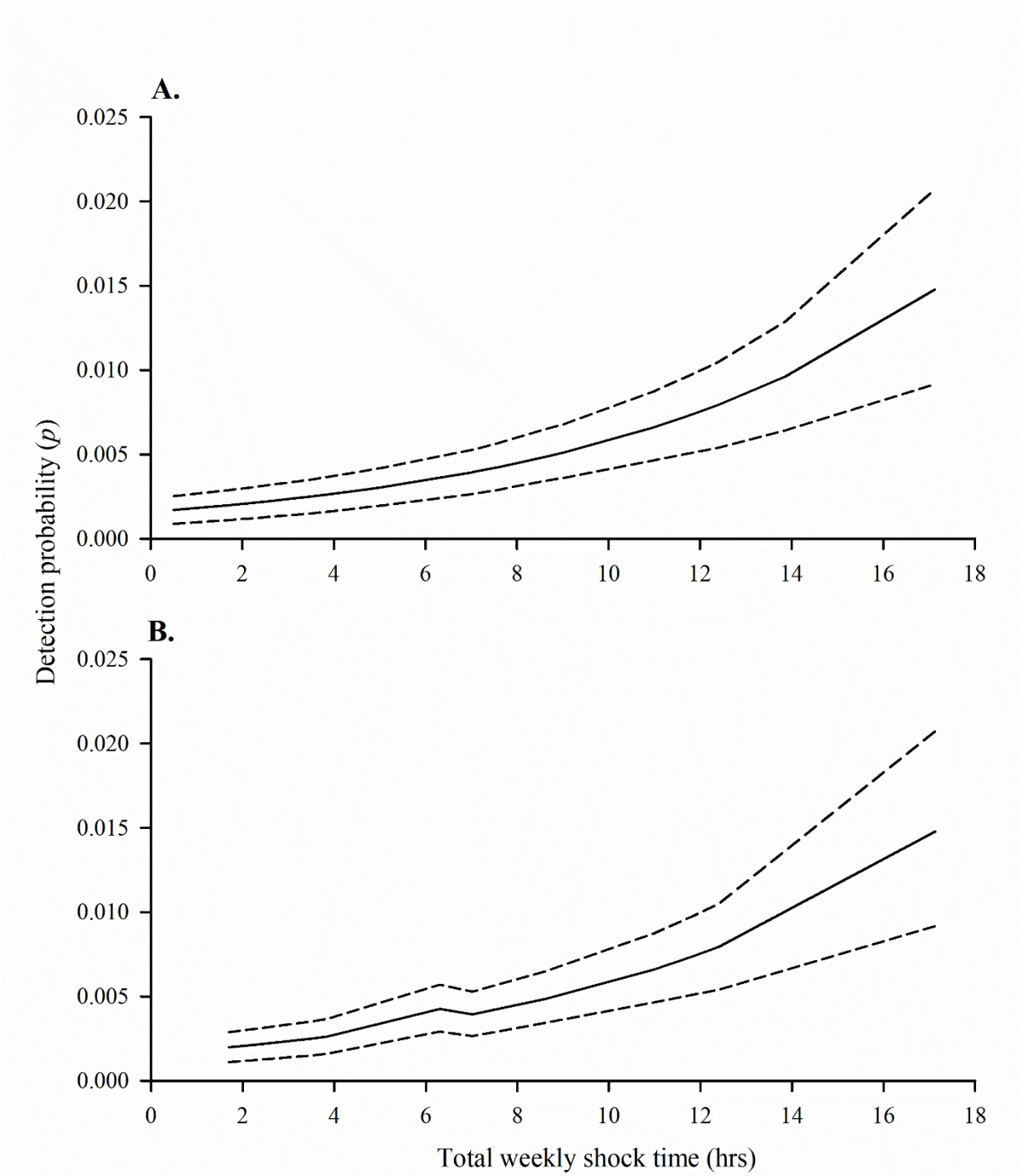

Figure 7.1 Mean detection probability (solid line; *p*) and 95% credibility intervals (dashed line) of initial stocking event (A) and second stocking event (B) as a function of total weekly boat electrofishing shock time (hrs).

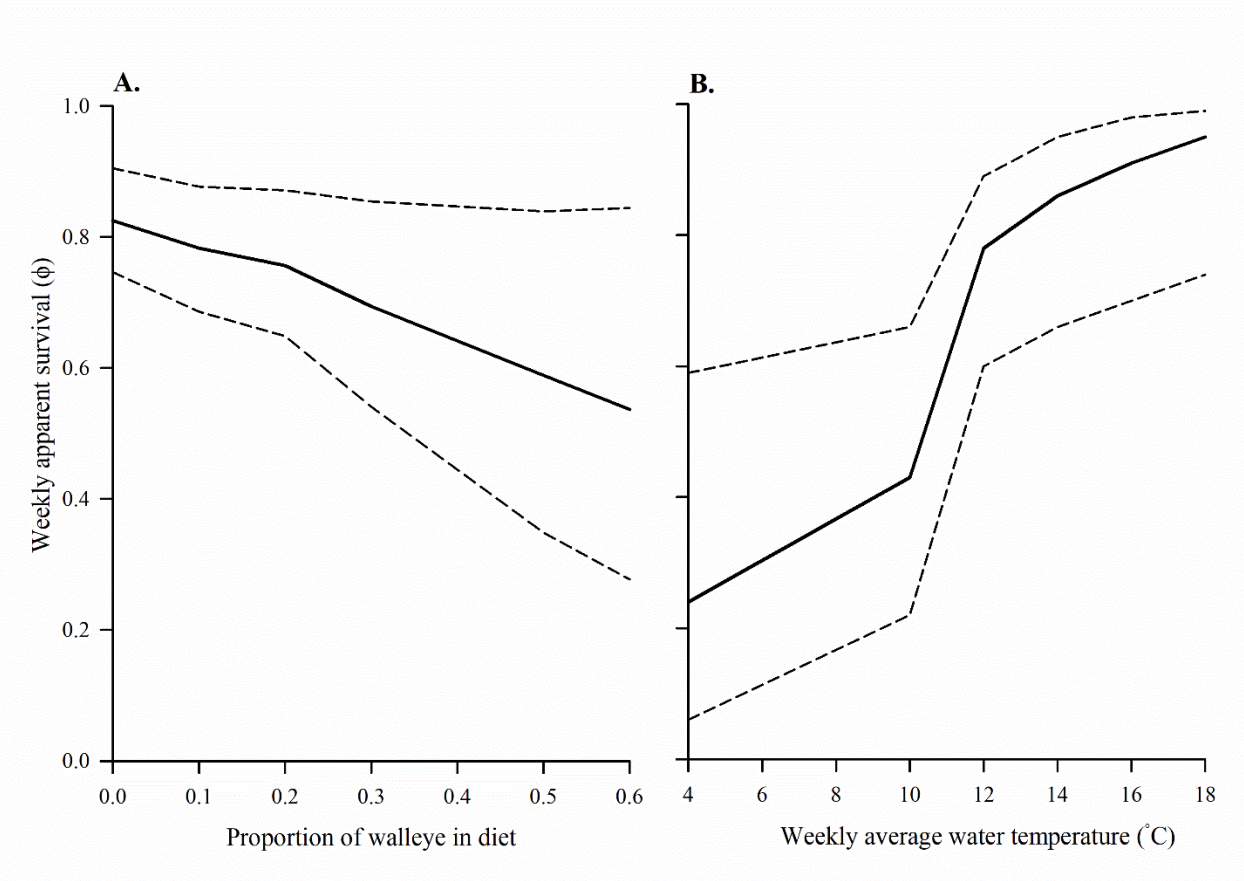

Figure 7.2 Mean apparent weekly survival (ϕ; solid line) and 95% credibility intervals (dashed lines) of walleye as a function of the weekly average proportion of walleye recovered from predator stomachs based on average weekly water temperature (13.2℃; A) and weekly average water temperatures (℃) based on the weekly average proportion of walleye in predator diets (0.11; B).

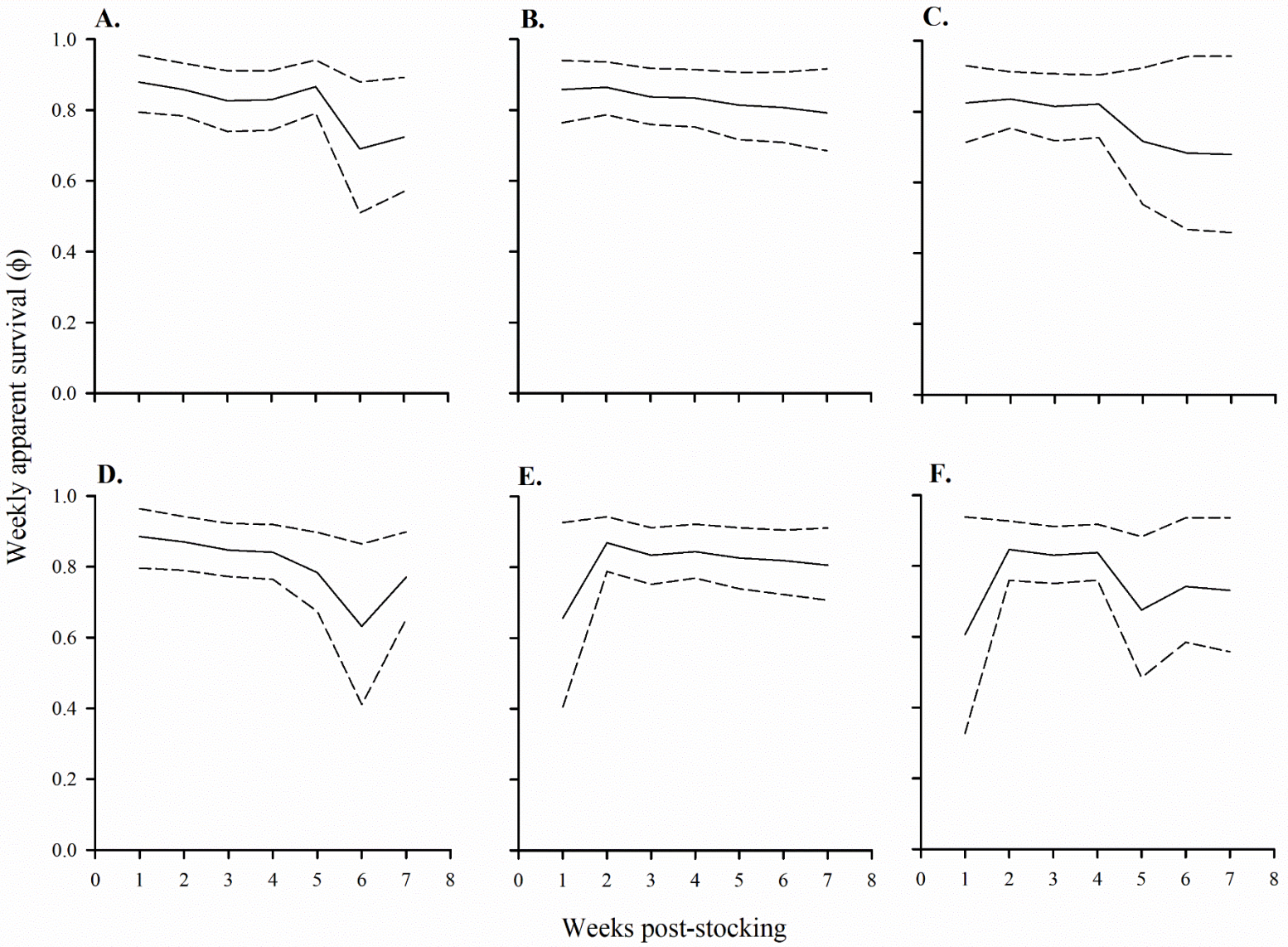
A.
B.
C.
D.
E.
F.
Weekly apparent survival (ϕ)
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8
Weeks post-stocking

Figure 7.3 Apparent weekly survival (ϕ) of walleye stocked into East Okoboji (top panels) and West Okoboji (bottom panels) during 2015 (A&D), 2016 (B&E), and 2017 (C&F). The solid line represents the mean, and dashed lines represent the 95% credibility intervals. Apparent weekly survival estimates vary across system and years, due to system and annual variability in the proportion of walleye in predator diets and weekly average water temperatures.

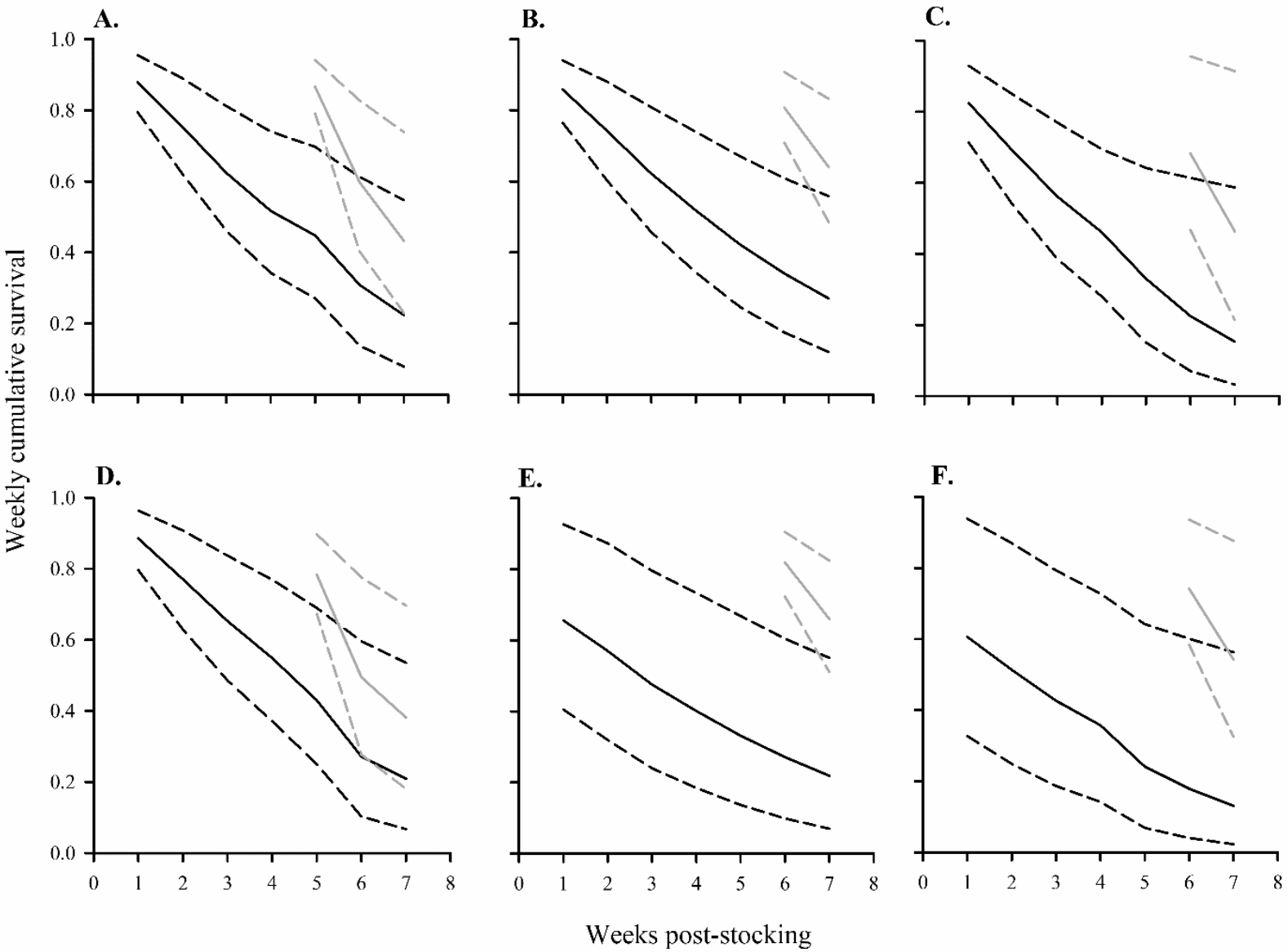
A.
B.
C.
D.
E.
F.
Weekly cumulative survival
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8
Weeks post-stocking

Figure 7.4 Cumulative apparent weekly survival of stocked walleye in East Okoboji (top row) and West Okoboji (bottom) row in 2015 (A&D), 2016 (B&E), and 2017 (C&F) relative to weeks post-stocking for Walleye stocked in two stocking events (September [back] and October [grey]). Solid lines represented the mean and dashed lines represent the 95% credibility intervals.

CHAPTER 8. GENERAL CONCLUSION

The practice of stocking fish as a fisheries management tool began in the 1800s (Jackson et al. 2004). Fish are stocked for a multitude of reasons that include establishing new angling opportunities, supplementing populations with limited recruitment, and biomanipulation (Wedemery 2001; Nickum et al. 2004; Barton 2011; Neal and Willis 2012). However, a challenge to stocking programs is variable post-stocking survival that can range from 0.00 to 0.99 (Coghlan et al. 2007; Steffensen et al. 2010; Zelasko et al. 2010; Weber et al. 2020). Thus, small changes in survival rates can result in significant differences in year-class strength and success of stocking initiatives. Therefore, understanding factors that limit stocking success of hatchery propagated fish is crucial for guiding fisheries management decisions and improving fisheries.

Numerous factors influence post-stocking survival of hatchery propagated fish including transportation and stocking mortality (Forsberg et al. 2001; Barton et al. 2003), predation (Stein et al. 1981; Murphy and Kelso 1986; Santucci and Wahl 1993; Buckmeier et al. 2005; Thompson et al. 2016), foraging ability (Szendrey and Wahl 1996; Kellison et al. 2000), competition (Le Pape and Bonhommeau 2015; Chase et al. 2016), body size (Litvak and Leggett 1992; Meekan et al. 2006; Grausgruber and Weber *in press*), and water temperatures (Hoxmeier et al. 2006; Akimova et al. 2016; Wagner et al. 2017). These factors do not act independently of each other. For example, abundances of prey and competitors can influence starvation and body condition (Santucci and Wahl 1993; Biro et al. 2004), fall energy reserves (Thompson et al. 1991; Garvey et al. 2004), and overwinter survival (Biro et al. 2004). Additionally, body size plays a critical role in predation risk, where increases in size are associated with decreased predation risk (Post and Evans 1989; Miranda and Hubbard 1994) due to improved maneuverability, swimming

speed (Videler 1993). Thus, larger individuals should have higher survival relative to smaller individuals. Ultimately, abiotic and biotic factors that regulate post-stocking survival can vary tremendously spatiotemporally as well as act independently or interact synergistically with one another, making processes regulating survival challenging to elucidate.

Walleye *Sander vitreus* is a highly sought-after sport fish with variable recruitment rates (Foust and Haynes 2007; Hansen et al. 2015, 2017). Thus, fisheries managers have supplemented Walleye populations with fingerling stocking initiatives (Hoxmeier et al. 2006; Kampa and Hatzenbelor 2009; Kerr 2011). However, fingerling Walleye can have highly variable stocking success (Fielder 1992; Jennings and Philipp 1992; Mitzner 1992; Kampa and Hatzenbelor 2009). Recently, fisheries management agencies started stocking progressively larger fingerling Walleye to circumvent processes that negatively influence the stocking success of smaller Walleye such as transportation practices (Fielder 1992; Clapp et al. 1997; Forsberg et al. 2001; Barton et al. 2003), predation (Santucci and Wahl 1993; Nate et al. 2003; Freedman et al. 2012; Kelling et al. 2016), and starvation (Szendrey and Wahl 1996; Kellison et al. 2000). However, little is known regarding the influence of these factors on the survival of larger fingerling Walleye (98-287 mm). The overarching objective of my dissertation was to use a multifaceted approach to identify and evaluating possible “bottlenecks” (i.e., stocking and transportation practices, predation, and starvation) hypothesized to limit large fingerling Walleye stocking initiatives. Specifically, I individually evaluated the effects of stocking and transportation practices (Chapters 2, 3), as well as post-stocking predation (Chapters 4, 5) and diet composition (Chapter 6) of fingerling Walleye. I then combined results from Chapters 2-6 and simultaneously evaluated the influence of all potential bottlenecks on fingerling Walleye apparent survival using

mark-recapture modeling (Chapter 7). In the following paragraphs, I will highlight significant findings from each chapter and conclude with future research directions.

An obstacle of stocking fish is unavoidable exposure to numerous handling and transport-related stressors (e.g., changes in water chemistry, transport duration, and crowding) that negatively influence health and survival, and limit stocking success (Huntingford et al. 2006). Transportation duration has been hypothesized to influence post-stocking survival of hatchery-reared fish due to its cascading effect on water quality parameters during transportation (*reviewed by* Sampaio and Freire 2016). Understanding the influence of transport duration and changes in water quality parameters during transportation can improve post-stocking survival of fingerling Walleye. Thus, the objective of Chapter 2 was to assess relationships between fingerling Walleye transport duration (3.5-6.0 hr) with changes in water chemistry parameters (dissolved oxygen, water temperature, and carbon dioxide), whole-blood glucose and plasma cortisol concentrations, and short-term (48-hr) mortality. I developed a set of candidate mixed linear models to evaluate relationships between whole blood glucose, plasma cortisol, and cumulative survival rates relative to water quality parameters observed during and after transportation (e.g., carbon dioxide, dissolved oxygen, and changes in water temperature) and transportation duration. Like prior research (Forsberg et al. 1999, 2001; Barton et al. 2003), I observed fluctuations in all water quality parameters as well as fingerling Walleye physiological parameters during and 48-hrs post-transport. Fingerling Walleye glucose concentration were influenced by carbon dioxide concentrations of transport water, while cortisol concentrations and cumulative survival rates were not related to changes in water quality parameters or transport duration, suggesting that characteristics unique to each transportation event influenced cortisol concentrations and cumulative 48-hr survival. Furthermore, fingerling Walleye transported 6

hours consistently exhibited post-transport lethargic behaviors associated with inhibited fight-or-flight responses. Overall, the results from Chapter 2 indicated that whole-blood glucose, plasma cortisol, and cumulative survival were not related to transport duration. However, fingerling Walleye transported any duration exhibited physiological responses and, in some cases, increased 48-hour cumulative mortality and altered behavioral responses. Based on our field evaluation, it became readily apparent that evaluating the effects of transportation and handling practices on physiological stress indicators (e.g., glucose and cortisol) in a field setting is challenging due to exposure to simultaneous transportation-related stressors (Barton 2000). Furthermore, challenges associated with field evaluations, and unavoidable inclusion of confounding factors such as multiple transportation dates (Carmichael 1984; Forsberg et al. 2001), stocking fish into different waterbodies with variable water quality parameters (Barton et al. 2003; Dobšíkova et al. 2006), and simultaneous changes of water quality parameters during transportation (e.g., pH, dissolved oxygen, carbon dioxide, and ammonia; Sampaio and Freire 2016) made it difficult to determine the effects of transportation duration on Walleye physiology and survival.

The objective of Chapter 3 was to develop an experimental approach that eliminated as many confounding factors from Chapter 2 as possible (e.g., changes in water quality parameters, stocking water characteristics, and multiple transportation days) to provide a more transparent evaluation of the effects of transportation duration on stress-related physiological changes and post-transportation mortality. For this evaluation, I developed a staggered loading protocol that allowed for all Walleye to be transported on the same truck on the same day and allowed us to keep fish densities in each of the three transportation truck compartments consistent throughout the experiment. Additionally, this approach allowed us to unload fish from each transportation

truck tank simultaneously, so they were exposed to the same post-transport conditions (Specker and Schreck 1980). Based on the results from Chapter 2, we increased the number of water quality parameters (dissolved oxygen, carbon dioxide, water temperature during transportation, transport water pH, unionized ammonia, total ammonia nitrogen, and total alkalinity) to evaluate whether any unmeasured parameters in Chapter 2 may have influenced physiological responses. Similar to our approach in Chapter 2, our analysis consisted of developing a set of candidate mixed linear models to evaluate relationships between whole blood glucose, plasma cortisol, and cumulative survival rates relative to water quality parameters during and after transportation (e.g., dissolved oxygen, carbon dioxide, water temperature during transportation, transport water pH, unionized ammonia, total ammonia nitrogen, and total alkalinity). Our top candidate models indicated that changes in Walleye physiological parameters (whole blood glucose and plasma cortisol) were not related to transportation duration, or water quality parameters (dissolved oxygen, carbon dioxide, water temperature during transportation, transport water pH, unionized ammonia, total ammonia nitrogen, and total alkalinity). However, unlike Chapter 2, fingerling Walleye transported different durations had high cumulative survival and no altered fight-or-flight responses. Collectively, results from Chapters 2 and 3 suggest that transporting fingerling Walleye results in prolonged elevated concentrations of whole blood glucose and plasma cortisol. Exposing fish to multiple stressors can have cumulative effects with those exposed to multiple stressors (e.g., handling and transportation) exhibiting changes in whole blood glucose and plasma cortisol concentrations as well as concentrations that remain elevated for longer durations of time (Barton et al. 1986). Therefore, stocking programs need to consider the number of stressors as well as stressor type (e.g., changes in water quality parameters and physical handling) and duration. Additionally, hatcheries should evaluate whether it is possible to

decrease the number of stressors during the loading and unloading processes. Ultimately, cumulative effects of stressors have the potential to influence fingerling Walleye behavior, making them more susceptible to predation (Freedman et al. 2012) and starvation (Santucci and Wahl 1993).

Post-stocking predation by piscivorous fishes can be a significant source of mortality for stocked fishes (Stein et al. 1981; Murphy and Kelso 1986). Walleye stocking success can be erratic, in part due to post-stocking predation (Fielder 1992; Jennings and Philipp 1992; Mitzner 1992). Largemouth Bass *Micropterus salmoides* (Santucci and Wahl 1993; Freedman et al. 2012; Kelling et al. 2016), Smallmouth Bass *Micropterus dolomieu* (Johnson and Hale 1977; Liao et al. 2004), Northern Pike *Esox lucius* (Anthony and Jorgensen 1977; Nate et al. 2003), Muskellunge *Esox masquinongy* (Bozek et al. 1999), and adult Walleye (Chevalier 1973; Liao et al. 2002) have been observed to consume age-0 Walleye and may negatively influence fingerling Walleye recruitment (Fayram et al. 2005). The growth–predation hypothesis predicts that selective mortality should decline as individuals grow and increase in size (Anderson 1988). Thus, larger individuals should have higher survival relative to smaller individuals. The objectives of Chapters 4 and 5 were to evaluate whether post-stocking predation was reliant on fingerling Walleye size and whether predation could consume a significant portion of a stocked fingerling Walleye population. In Chapter 4, I assessed whether consumed fingerling Walleye total length was related to predator total length, predator gape height, or the probability of predation and if total length distributions varied among stocked, recaptured, and consumed fingerling Walleye. Results from Chapter 4 indicated mean, maximum (85th percentile), and minimum (15th percentile) consumed fingerling Walleye total length was not related to predator total length or gape height, but the probability of predation decreased by 0.02 for every 10 mm increase in

fingerling Walleye total length. Comparisons of stocked, recaptured, and consumed fingerling Walleye total length distributions indicated consumed fingerling Walleye were generally smaller whereas recaptured fingerling Walleye tended to be larger than stocked fish. To further understand the effects of post-stocking predation on stocked fingerling Walleye, I used bioenergetics modeling to determine the biomass of consumed fingerling Walleye from stocking through ice-up (Chapter 5). Results from Chapter 5 suggest that Largemouth Bass, Northern Pike, and adult Walleye collectively consumed between 2.4-27.2% of the stocked fingerling Walleye within two months of stocking, with predators consuming higher proportions of smaller (<220 mm) fingerling Walleye. All predators exhibited temporal changes in the proportion of fingerling Walleye in their diets. Specifically, in East Okoboji, the highest proportions of fingerling Walleye in predator diets generally occurred 14 days after the most recent stocking event with the proportion of fingerling Walleye in predator diets decreasing thereafter. However, in West Okoboji, Northern Pike, adult Walleye, and Largemouth Bass had increasing proportions of fingerling Walleye in diets between stocking events. Collectively, results from Chapters 4 and 5 indicate that morphological features (total length and gape height) of predators were not directly related to consumed fingerling Walleye total length and predators consume 2.4-27.2% of stocked fingerling Walleye with higher consumption of smaller fingerling Walleye (< 220 mm). From an economic standpoint, post-stocking predation can have financial ramifications. Stocking additional fingerling Walleye could offset losses due to post-stocking predation or stocking fingerling Walleye at sizes rarely consumed by predators (≥ 90g; TL = 220 mm) could result in improved post-stocking survival by decreasing the number of fingerling Walleye consumed by predators. Both options may result in added hatchery production costs. Furthermore, stocked fishes are occasionally placed in acclimation cages for 3-6 days to decrease post-stocking

predation (Jonsson et al. 1999; Brennan et al. 2006) but would not likely be beneficial in situations where post-stocking predation is prolonged as observed by predators. Alternatively, predator recognition training during the rearing process can condition fish to recognize chemical and visual cues of predators and has shown promise in decreasing post-stocking predation (Brown and Smith 1998; Brown and Laland 2001). Therefore, stocking fingerling Walleye ≥ 220 mm TL, stocking additional fingerling Walleye, as well as the use of acclimation cages or predator training all have the potential to improve fingerling Walleye stocking success in systems that historically had erratic post-stocking survival, partially due to post-stocking predation by a suite of predators.

Since the 1980s, there has been a growing concern as to whether hatchery fish lack natural foraging behaviors (Sosiak et al. 1979; Johnsen and Ugedal 1989; Sundstom and Johnsson 2001) in part due to rearing practices where fish are raised in tanks void of habitat and predators, as well as habituation to pellets. Studies evaluating post-stocking feeding behaviors of hatchery-reared fish have had varying results that differ across species. There have been several laboratory evaluations assessing the effects of pellet rearing on fingerling Walleye diet composition (Wahl et al. 1995; Wisenden et al. 2004); yet there are limited field evaluations. In Chapter 6, I compared fall dietary habits of wild and fingerling stocked Walleye and evaluated the influence of total length on the presence of prey items in their diets. Overall, we observed differences in diet composition of wild and fingerling Walleye. Specifically, fingerling Walleye had higher average (± 95% CI) proportions of empty stomachs relative to wild conspecifics (wild = 0.15 ± 0.09; fingerling = 0.40 ± 0.10). Both wild and fingerling Walleye exhibited temporal trends in diet composition with decreasing proportions zooplankton and increasing proportions of benthic invertebrates and fish. Percent similarity index values suggest that wild and fingerling

Walleye diets were initially similar (PSI = 0.60) and then decreased. However, after a month, the diet composition of the two cohorts became increasingly similar (PSI = 0.61). Wild and fingerling Walleye total length had little effect on the presence of zooplankton, benthic invertebrates, and fish in diets. Collectively, our results suggest that pellet training fingerling Walleye may alter post-stocking diet composition, which could negatively influence post-stocking survival by increasing starvation as well as promoting feeding behaviors (e.g., staying high in the water column during feeding) that could increase post-stocking predation. Previous research has suggested that feeding hatchery fish live forage can improve capture success, diet composition, color, and size at stocking (Colgan et al. 1986; Ibrahim and Huntingford 1992; Szendrey and Wahl 1995; Larscheid et al. 1999; Ellis et al. 2002). Thus, transitioning fingerling Walleye to live prey in hatcheries for a short amount of time before stocking may improve their ability to capture live prey after stocking. Furthermore, supplementing diets with live forage before stocking can reduce feeding behaviors, such as staying high in the water column for long durations, which can result in increased chances of post-stocking predation (Suboski and Templeton 1989). Incorporation of life skills training (exposure to life forage) before stocking could improve fingerling Walleye stocking success.

In Chapters 2-6, I evaluated individual parameters hypothesized to influence stocked fingerling Walleye survival, such as the effects of transportation (Chapters 2, 3) as well as post-stocking predation (Chapters 4,5), and diet composition (Chapter 6). While this approach provided useful information, it was limited in its ability to rank the importance of each parameter. Mark-recapture modeling provided a methodology for comparing the individual and additive effects of parameters evaluated in Chapters 2-6. In Chapter 7, I used mark-recapture techniques and modeling to assess the influence of predation (proportions of fingerling Walleye

in predator diets, total predator [Largemouth Bass, Muskellunge, Northern Pike, Smallmouth Bass, and adult Walleye] relative abundance, Largemouth Bass relative abundance, Northern Pike relative abundance, and variety of prey types in predator diets), characteristics specific to the stocked fingerling Walleye (total length and condition at time of stocking and proportion of Walleye with empty diets), and stocking environment (water temperature at time of stocking and average weekly water temperatures) on fall apparent weekly survival of fingerling Walleye. Cormack-Jolly-Seber recapture models estimated that apparent survival was negatively related to the weekly average proportion of fingerling Walleye recovered from predator diets (β = -2.86; 95% credibility interval = -5.69 to -0.17). There was some evidence that weekly average water temperatures influenced apparent survival (β = 0.09; 95% credibility interval = -0.08 to 0.20); however, the 95% credibility interval overlapped zero. Mean apparent weekly survival ranged from 0.75 to 0.91 when no fingerling Walleye were recovered from predator diets and 0.28 to 0.88 when predator diets contained various proportions of fingerling Walleye. Cumulative survival indicated that 41-97% of stocked fingerling Walleye were lost to mortality from stocking through ice-up, indicating that the two months post-stocking is a critical period for survival. Overall, our results suggest that post-stocking fingerling Walleye survival is most influenced by post-stocking predation rather than characteristics specific to stocked fingerling Walleye or environmental factors. Assessing post-stocking survival rates of hatchery fishes is essential to understanding and potentially identifying potential sources of mortality that can result in recruitment success or failure. Fingerling Walleye apparent survival was negatively related to predator consumption of fingerling Walleye, suggesting that post-stocking predation is a primary factor that influences stocking success. Moreover, the lack of support for factors related to environment and characteristics of stocked fingerling Walleye suggests that fisheries

managers managing systems with erratic recruitment of stocked fingerling Walleye should initially consider focusing on decreasing post-stocking predation, such as life skills training, post-transport acclimation cages, or stocking areas with low predator densities.

The scientific inquiry process is an iterative process resulting in the evolution of new questions and experimental evaluations. My dissertation is no exception, in that each of my chapters has resulted in a substantial advancement of the knowledge of factors affecting fingerling Walleye post-stocking survival but has also raised additional research questions. In regard to the influence of transportation on fingerling Walleye stress and survival, future work should attempt to understand why fingerling Walleye transported 6 hours exhibited altered fight-or-flight responses. Additionally, future transportation research should decipher which components of the loading process (e.g., seining, pushing across the sorting table, crowding during the weighing process, etc.) elicit significant physiological responses. While our staggered loading protocol decreased the number of confounding factors, our experimental design resulted in pseudoreplication because the experimental unit was individual transport durations, which was only done once. Thus, future evaluations should try this approach multiple times and use each transport date as a replicate. With respect to my research regarding the influence of post-stocking predation (Chapters 4, 5) and fingerling Walleye diet shifts (Chapter 6), results suggest that life skills training before stocking may result in decreased post-stocking predation as well as increases in diet composition that is similar to wild conspecifics. There is a vast amount of literature on life skills training of hatchery propagated salmonid species (Jaervi and Uglem 1993; Berejikian et al. 1999; Mirza and Chivers 2000; Hawkins et al. 2008) and species of conservation concern (Archer and Crowl 2014; Wishingrad et al. 2014; Sloychuk et al. 2016); yet, there has been very little research about percid species, such as Walleye (*but see* Wisenden et al. 2004).

Thus, future research should consist of experimental and field evaluations as to the effectiveness of life skills training as it is related to post-stocking predation and diet composition. Based on the overall dissertation results, fisheries biologists managing systems stocked with Walleye that exhibit erratic recruitment, such as East Okoboji and West Okoboji, should consider stocking alternative locations, such as stocking Walleye off boat ramps that are not close to deep weed beds where predators may have abundant densities as well as consider stocking Walleye from boats which would offer more flexibility in location and number of potential stocking locations. Additionally, fisheries management agencies that transport Walleye six or more hours may want to consider reevaluating where Walleye are produced in effort to decrease transport duration to under six hours. Furthermore, aquaculture facilities rearing Walleye should consider incorporating and evaluating life skills training into Walleye propagation. Specifically, exposure to live forage prior to stocking could improve both post-stocking diet composition as well as decrease feeding behaviors that may increase stocked Walleye vulnerability to predation. Although several questions are still unanswered, this dissertation provides a strong foundation for future assessments regarding the identification and evaluation of bottlenecks that could be influencing the success of fingerling Walleye stocking programs.

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APPENDIX. BIOENERGETICS PREDATOR DIET DATA

Daily proportions by weight (g) of Ictaleuridae, Centrarchidae, Decapoda, Cyprinidae, other fish, Sciaenidae, adult Anura, Clupeidae, Insecta, larval Anura, unidentifiable, age-0 Walleye, Moronidae, and Percidae consumed by Largemouth Bass, Northern Pike, and adult Walleye from East Okoboji and West Okoboji lakes, Iowa, USA during 2016 and 2017. 'Day' indicates days in which predator diets were collected in each lake and year and were used in the bioenergetics models.

Day	Prey Categories													
	Ictaleuridae	**Centrarchidae**	**Decapoda**	**Cyprinidae**	**Other fish**	**Sciaenidae**	**Adult Anura**	**Clupeidae**	**Insecta**	**Larval Anura**	**Unidentifiable**	**Age-0 Walleye**	**Moronidae**	**Percidae**
Largemouth Bass: 2017 East Okoboji														
1	0.00	0.00	0.00	0.17	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.17	0.00
2	0.00	0.26	0.00	0.00	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.16	0.00
6	0.00	0.67	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	0.05	0.33	0.07	0.00	0.30	0.00	0.00	0.00	0.00	0.01	0.00	0.07	0.17	0.00
10	0.06	0.31	0.03	0.00	0.25	0.03	0.06	0.00	0.00	0.00	0.03	0.09	0.11	0.03
12	0.06	0.28	0.11	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.11	0.17	0.06	0.00
34	0.50	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
36	0.33	0.00	0.00	0.00	0.33	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
37	0.00	0.00	0.33	0.00	0.04	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00
39	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.33	0.00
41	0.00	0.00	0.17	0.00	0.50	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.17	0.00
Largemouth Bass: 2017 West Okoboji														
1	0.00	0.00	0.18	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.65	0.00	0.00
3	0.00	0.13	0.20	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.13	0.09
7	0.05	0.05	0.42	0.00	0.14	0.00	0.05	0.00	0.00	0.00	0.05	0.26	0.00	0.00
11	0.00	0.24	0.25	0.00	0.08	0.00	0.08	0.00	0.00	0.10	0.00	0.15	0.00	0.09
13	0.17	0.00	0.54	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00
20	0.00	0.07	0.20	0.04	0.33	0.00	0.04	0.00	0.16	0.00	0.00	0.09	0.00	0.06
28	0.00	0.41	0.03	0.00	0.40	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00
31	0.00	0.23	0.22	0.04	0.25	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00
35	0.00	0.00	0.04	0.00	0.00	0.00	0.96	0.00	0.00	0.00	0.00	0.00	0.00	0.00
38	0.02	0.02	0.22	0.00	0.16	0.00	0.34	0.00	0.00	0.02	0.00	0.18	0.02	0.00
40	0.00	0.06	0.21	0.00	0.19	0.00	0.34	0.00	0.00	0.00	0.08	0.12	0.00	0.00
42	0.02	0.00	0.10	0.00	0.08	0.00	0.47	0.00	0.00	0.04	0.00	0.27	0.00	0.02
45	0.00	0.18	0.05	0.00	0.28	0.00	0.19	0.00	0.00	0.07	0.00	0.18	0.05	0.00
Largemouth Bass: 2016 East Okoboji														
1	0.00	0.37	0.00	0.00	0.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00
3	0.08	0.29	0.00	0.00	0.52	0.04	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00
8	0.00	0.36	0.00	0.00	0.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
14	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
35	0.00	0.17	0.00	0.00	0.36	0.14	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Day	Prey Categories													
	Ictaleuridae	Centrarchidae	Decapoda	Cyprinidae	Other fish	Sciaenidae	Adult Anura	Clupeidae	Insecta	Larval Anura	Unidentifiable	Age-0 Walleye	Moronidae	Percidae
37	0.00	0.19	0.05	0.00	0.39	0.06	0.10	0.00	0.00	0.00	0.00	0.00	0.19	0.01
39	0.05	0.18	0.05	0.00	0.56	0.05	0.05	0.00	0.02	0.00	0.05	0.00	0.00	0.00
41	0.00	0.00	0.17	0.00	0.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
42	0.00	0.27	0.03	0.00	0.24	0.03	0.13	0.00	0.00	0.00	0.00	0.03	0.25	0.03
48	0.00	0.08	0.00	0.08	0.38	0.00	0.38	0.00	0.00	0.00	0.00	0.00	0.08	0.00
50	0.00	0.13	0.00	0.00	0.40	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.07	0.00
Largemouth Bass: 2016 West Okoboji														
1	0.00	0.17	0.02	0.00	0.47	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.14	0.00
3	0.05	0.06	0.13	0.00	0.05	0.00	0.01	0.00	0.00	0.00	0.00	0.67	0.00	0.04
5	0.10	0.07	0.15	0.00	0.35	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.00	0.00
7	0.00	0.29	0.33	0.03	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.00	0.00
14	0.11	0.22	0.44	0.00	0.11	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	0.00	0.17	0.17	0.00	0.50	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00
37	0.00	0.23	0.10	0.00	0.23	0.00	0.33	0.00	0.02	0.04	0.00	0.00	0.00	0.06
39	0.03	0.24	0.15	0.00	0.31	0.03	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.05
41	0.00	0.11	0.09	0.00	0.32	0.00	0.28	0.00	0.00	0.07	0.00	0.07	0.00	0.07
44	0.00	0.32	0.10	0.00	0.27	0.00	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.01
50	0.17	0.28	0.00	0.00	0.09	0.00	0.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Northern Pike: 2017 East Okoboji														
1	0.00	0.10	0.00	0.00	0.18	0.00	0.00	0.10	0.00	0.00	0.00	0.40	0.12	0.10
2	0.00	0.26	0.00	0.05	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.11	0.05
6	0.13	0.25	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.38	0.00
8	0.00	0.00	0.00	0.08	0.18	0.00	0.00	0.00	0.00	0.08	0.00	0.46	0.06	0.15
10	0.00	0.43	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.15	0.08
12	0.00	0.36	0.00	0.00	0.64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
34	0.00	0.65	0.00	0.00	0.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
36	0.00	0.30	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.34	0.00
37	0.00	0.21	0.04	0.00	0.24	0.07	0.04	0.00	0.00	0.00	0.00	0.00	0.40	0.00
39	0.00	0.26	0.00	0.01	0.22	0.05	0.03	0.00	0.00	0.01	0.00	0.11	0.26	0.04
41	0.00	0.39	0.00	0.00	0.14	0.08	0.00	0.00	0.00	0.00	0.00	0.11	0.28	0.00
Northern Pike: 2017 West Okoboji														
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
7	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	0.00	0.17	0.00	0.00	0.54	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.08
13	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	0.00	0.27	0.00	0.00	0.15	0.00	0.13	0.00	0.00	0.00	0.13	0.06	0.00	0.27
28	0.00	0.34	0.00	0.00	0.36	0.02	0.00	0.06	0.00	0.00	0.03	0.00	0.12	0.06
31	0.00	0.61	0.00	0.00	0.05	0.00	0.17	0.00	0.00	0.00	0.00	0.10	0.00	0.06
38	0.00	0.23	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00
40	0.00	0.33	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00
42	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
45	0.00	0.63	0.00	0.00	0.11	0.00	0.04	0.00	0.00	0.06	0.00	0.13	0.00	0.04
Northern Pike: 2016 East Okoboji														
1	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00
3	0.00	0.18	0.00	0.02	0.39	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.08	0.00
8	0.00	0.31	0.00	0.00	0.67	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.38	0.00
35	0.10	0.13	0.00	0.00	0.40	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00
37	0.00	0.32	0.00	0.00	0.33	0.26	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00
39	0.00	0.08	0.00	0.00	0.47	0.35	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00
41	0.00	0.00	0.00	0.00	0.20	0.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
42	0.00	0.20	0.00	0.00	0.60	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Day	Prey Categories													
	Ictaleuridae	Centrarchidae	Decapoda	Cyprinidae	Other fish	Sciaenidae	Adult Anura	Clupeidae	Insecta	Larval Anura	Unidentifiable	Age-0 Walleye	Moronidae	Percidae
48	0.00	0.07	0.00	0.06	0.30	0.40	0.06	0.00	0.00	0.02	0.00	0.00	0.10	0.00
50	0.00	0.00	0.00	0.00	0.58	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Northern Pike: 2016 West Okoboji														
1	0.00	0.30	0.00	0.00	0.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.04	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.86	0.00	0.00
5	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.88	0.00	0.00
9	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
16	0.00	0.43	0.00	0.00	0.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
37	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.33	0.17
39	0.00	0.40	0.00	0.00	0.40	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.14
41	0.00	0.00	0.00	0.00	0.50	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
44	0.00	0.38	0.00	0.00	0.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50	0.00	0.36	0.00	0.00	0.41	0.08	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.06
54	0.00	0.50	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Adult Walleye: 2017 East Okoboji														
1	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.16	0.00	0.00	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00
6	0.00	0.17	0.00	0.17	0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.00
8	0.00	0.29	0.00	0.00	0.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00
10	0.00	0.42	0.00	0.00	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.00
12	0.00	0.18	0.00	0.00	0.48	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.09
36	0.00	0.35	0.00	0.00	0.15	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.28	0.11
37	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.95	0.00
39	0.00	0.00	0.00	0.00	0.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.39	0.00
41	0.00	0.05	0.00	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.00
Adult Walleye: 2017 West Okoboji														
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
7	0.00	0.35	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.36	0.09	0.00
11	0.09	0.40	0.00	0.00	0.51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	0.00	0.55	0.00	0.00	0.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	0.00	0.37	0.08	0.00	0.43	0.00	0.05	0.00	0.00	0.00	0.00	0.07	0.00	0.00
28	0.00	0.33	0.00	0.04	0.35	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.05
31	0.00	0.72	0.00	0.00	0.20	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.03	0.00
35	0.00	0.00	0.00	0.00	0.44	0.00	0.00	0.00	0.00	0.56	0.00	0.00	0.00	0.00
38	0.09	0.17	0.00	0.00	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.17	0.08
40	0.02	0.33	0.07	0.01	0.32	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.07	0.00
42	0.00	0.28	0.00	0.00	0.47	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.13	0.00
45	0.00	0.38	0.00	0.00	0.27	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.08	0.13
Adult Walleye: 2016 East Okoboji														
1	0.00	0.51	0.00	0.00	0.49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.32	0.00	0.00	0.53	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00
5	0.00	0.38	0.00	0.00	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00
8	0.00	0.24	0.00	0.00	0.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.04
14	0.00	0.49	0.00	0.00	0.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.05
35	0.00	0.05	0.00	0.00	0.53	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.00
37	0.04	0.04	0.00	0.00	0.65	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.03
39	0.00	0.11	0.00	0.00	0.78	0.06	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
41	0.00	0.14	0.00	0.00	0.33	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00
42	0.00	0.17	0.00	0.00	0.63	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05

Day	Prey Categories													
	Ictaleuridae	**Centrarchidae**	**Decapoda**	**Cyprinidae**	**Other fish**	**Sciaenidae**	**Adult Anura**	**Clupeidae**	**Insecta**	**Larval Anura**	**Unidentifiable**	**Age-0 Walleye**	**Moronidae**	**Percidae**
48	0.00	0.00	0.10	0.00	0.47	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00
50	0.00	0.08	0.00	0.00	0.31	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.42	0.00
Adult Walleye: 2016 West Okoboji														
1	0.00	0.33	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.13	0.05	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00
5	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.75	0.00	0.00
7	0.00	0.32	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.08
14	0.00	0.27	0.00	0.02	0.71	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	0.00	0.34	0.00	0.00	0.52	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
37	0.00	0.60	0.00	0.00	0.21	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00
39	0.00	0.52	0.00	0.04	0.36	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
41	0.00	0.35	0.00	0.20	0.33	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05
44	0.00	0.47	0.00	0.03	0.33	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00
50	0.00	0.30	0.00	0.06	0.24	0.11	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.07